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STUDIES IN NATURAL HYBRIDIZATION OF WHEAT[1]

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INTRODUCTION

The wheat plant is normally self-fertilized. Numerous instances of natural cross-fertilization, however, have been recorded. These include crosses between wheats of the same or different species, between wheat and rye, and between wheat and Aegilops. The amount of such natural crossing has usually been so small in the aggregate that special preventive measures have not been considered necessary in ordinary investigations with the wheat plant.

In the studies of Nilsson-Ehle (*8*) [2] the highest percentage of natural hybrids found in any wheat variety was 0.9 per cent. At least 2 to 3 per cent of natural crossing for several years at the Minnesota Agricultural Experiment Station is reported by Hayes (*3*) and Hayes and Garber (*4*). Garber and Quisenberry (*2*) report a maximum of 0.5 per cent of F_1 plants in the Mammoth Red variety grown at the West Virginia station.

Natural crossing in partially sterile species hybrids may be more frequent than in ordinary wheat. About 18.5 per cent of the F_3 plants grown by Hayes, Parker, and Kurtzweil (*5*), in certain families of crosses between *Triticum vulgare* and *T. durum*, were natural hybrids, evidently due to crossing in the F_2. In such partially sterile hybrids, unfertilized flowers may remain open for several days, thus allowing easy access of foreign pollen.

Natural wheat-rye hybrids have been reported several times. The most frequent occurrence was reported by Meister (*7*) at the Saratov (Russia) Experimental Station, where about 20 per cent of the plants in certain winter-wheat plots were natural wheat-rye hybrids in 1918. Leighty and Sando [3] found about 18 per cent natural wheat-rye hybrids in a Chinese variety at Arlington Experiment Farm in 1925.

Popova (*9*) reports finding hundreds of natural F_1 hybrids between wheat and Aegilops in 1921 and 1922 near Tashkent, Turkestan, and in 1922 also near Jan Aryk.

Numerous other instances of natural crossing in wheat have been reported, but an exhaustive review will not be made here. Many of the reports are of one or a few hybrid plants that have been found, while in other cases several such plants are recorded.

[1] Received for publication May 26, 1927; issued December, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 887.

[3] LEIGHTY, C. E., and SANDO, W. J. NATURAL AND ARTIFICIAL HYBRIDS OF A CHINESE WHEAT AND RYE. Jour. Heredity. (In press.)

HOW HYBRIDS OCCUR AND HOW RECOGNIZED

At blooming time the glumes of the wheat flower usually open and anthesis occurs in such a manner that a portion or at times possibly all of the pollen from the three anthers is shed into the air. This permits the cross-pollination of other flowers through the liberated pollen falling upon them or being carried to them by the wind. Flowers for any reason devoid of pollen are especially subject to cross-fertilization. Any condition, then, that results in a lack of pollen in some flowers may be conducive to natural crossing. Disease infection, the adaptation of the variety, climatic and soil conditions, or other conditions of the environment may influence pollen production and may thus be factors determining the degree of natural crossing.

The detection of natural hybridization is facilitated if it occurs between plants with strongly contrasted characters. It is not apparent when it occurs between plants of a single homogeneous variety. The greater the number of varieties with contrasted characters grown in proximity to one another, then the more likely is natural crossing to be recognized. The grouping in the nursery of varieties blooming at the same time, as is often done to lessen the difficulties of harvesting, facilitates crossing among them.

In wheat hybrids the factors for certain head characters are dominant, or partly so, to their allelomorphs. Thus the factors for absence of awns, red glumes, and pubescent glumes are more or less dominant to the factors for presence of awns, white glumes, and glabrous glumes, respectively. If a variety having one or more of the recessive factors is the female parent in a cross with a variety having one or more of the dominant factors, the hybrid is recognizable in the F_1. In the reciprocal cross in which the variety having the dominant factor is the female parent, the F_1 would not be recognized as a rule. In a variety of the Fulcaster type, which is awned and has white, glabrous glumes, the three principal recessive head characters are combined. Any plant, then, with one or more of the allelomorphic dominant head characters found in a variety of the Fulcaster type might be suspected of being a natural hybrid. For this reason, natural crossing may appear to occur more frequently in a variety having the recessive characters.

MATERIAL AND METHODS

The purpose of this paper is to report the results of observations and experiments on natural crossing in wheat at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., during the period 1917 to 1926. The degree of cross-pollination disclosed by these investigations has been higher than that previously reported by others. In fact, sufficient cross-fertilization between varieties has occurred to demonstrate the necessity of special technic in the conduct of certain agronomic or other investigations when purity of plant material is essential. Some information also has been accumulated relating to the causes of natural crossing.

More than 5,000 nursery rows and from 200 to 300 plots usually are grown each year at the Arlington Experiment Farm. These include most of the botanical types of common wheat as well as

representatives of the different wheat species. Many exotic forms unadapted to this locality are included.

The different varieties and strains are usually arranged in the nursery with reference to their time of ripening and habit of growth, in order to facilitate harvesting and equalize competition between adjacent rows. Very diverse morphological types, however, are often grown side by side or end to end. In 1917 and 1918, when some of the work here reported was done, the different strains were grown in single rows 18 feet long and 12 inches apart. About 124 such rows made up a block or series (Fig. 1.) Each block was separated from the next by a 2-foot path or alley. The arrangement of the varieties grown in plots is based largely on taxonomic characters. Some consideration is given to time of ripening, but this is secondary, and adjacent varieties are usually dissimilar in some major head character. This method is followed to prevent accidental mixing in harvesting and threshing. The field plots are one-fortieth of an acre in size (132 feet long by 8.25 feet wide) with an 18-inch alley between plots. Three replications of each variety are usually grown, and the entire plot is harvested for yield determination.

Many of the varieties grown in the nursery are unadapted to eastern wheat culture. Most of these are discarded after being tested from one to three years, but others are continued as breeding stock or for other special purposes. The varieties grown in plots are largely adapted types, though such partially unadapted varieties as Kanred and Nebraska No. 28 may be continued in test for periods of three to five or more years.

CLIMATIC AND GROWING CONDITIONS

The relationship between climate and natural crossing in wheat has been suggested by Howard and Howard (*6*), who believe that a dry, hot climate is conducive to cross-fertilization. The Arlington Experiment Farm has a decidedly humid climate, the 15-year (1912–1926) average annual precipitation being 40.57 inches for the crop year, July to June, inclusive. For the three months of active growth, April, May, and June, the average rainfall for this 15-year period was 11 inches.

The soil type is classed as Keyport silt loam. By the free use of soiling crops of rye and cowpeas, the occasional spreading of manure, and the application of acid phosphate to the grain crops, a high degree of productivity has been developed.

Straw and grain yields are high, and the tillering of the adapted varieties is above the average. The production of several tillers by each plant extends the blooming period of the variety. In most seasons the secondary tillers do not develop fully, and the fertility of these later heads is often poor. The most common disease of wheat is leaf rust (*Puccinia triticina* Erikss.), but this usually develops late in the season and therefore has little effect on the flowering or seed set of the crop. Other diseases, particularly Septoria, scab (*Gibberella saubinetii* (Mont.) Sacc.), and black chaff (*Bacterium translucens undulosum* EFS.), though less prevalent than leaf rust, may possibly exert some influence on the seed set of wheat.

NATURAL WHEAT HYBRIDS IN 1918

The seed for the 1918 rod rows was handled with unusual care in the 1917 harvest. After 16 feet had been cut from the 18-foot rows for yield determinations, heads typical of the variety in the row were

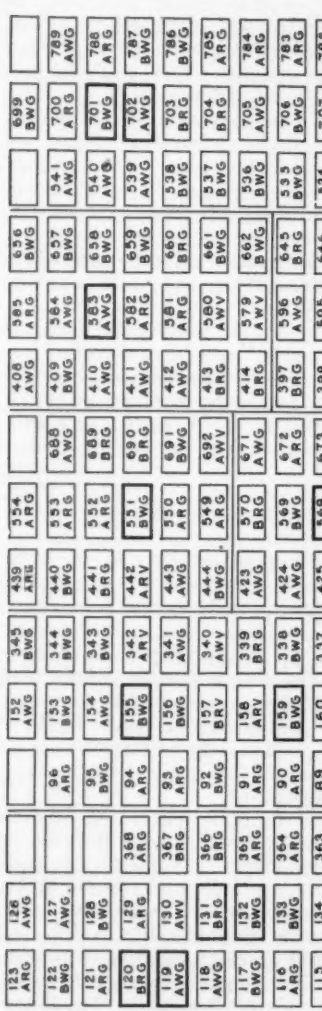

FIG. 1.—Diagram showing location and head characters of the nursery rows in 1917 (heavy lines), seed from which produced the natural hybrids selected in 1918, and the head characters of the near-by rows. The rows were 18 feet long and 12 inches apart. Each series was separated from the next by a 2-foot alley

gathered in separate containers from the remaining 2 feet at the ends. These heads were examined again later and threshed in a plant thresher, in which machine mixture is practically eliminated. The seed obtained was sown by hand in the fall of 1917, but the rows were

covered by a wheel hoe, which may occasionally carry a seed across the alley to the opposite row—especially when the soil is damp—or bring over a seed from an adjoining row.

Despite these efforts to maintain the different strains in pure condition, a surprising number of off-type plants were present in certain rows of the 1918 crop. Many of these apparently were F_1 hybrids resulting from natural crosses with strains grown in adjoining or near-by rows the previous year. In order to test this supposition, a head from each of the off-type plants present in the rows of 19 varieties or strains showing impurity in the 1918 crop was harvested and threshed by hand. The kernels from each head were space planted in head rows the following fall, and data on the head characters of each plant were obtained in 1919. These data are given in Table 1.

TABLE 1.—*Varieties of wheat grown in 18-foot rows at Arlington Experiment Farm in 1918 in which off-type plants were found, with head characters of the varieties and off-type plants, the probable origin of the off-type plants based on their breeding behavior, and the percentage of natural F_1 hybrids from 1917 seed*

Row No. in 1918	Variety	C. I. No.	Head characters of variety[a]	Off-type plants in 1918 rows of the variety		Breeding behavior of off-type plants in 1919 (number of plants with given head characters)[a]								Probable origin of the off-type plants[b]	Number of plants grown in 1919 from 1917 seed remnants		Percentage of F_1 hybrids from 1917 seed
				Number	Head characters[a]	ARG	AWG	BRG	BWG	ARV	AWV	BRV	BWV		Total	Number with head characters like F_1 hybrids in 1918 rows	
119	Fultzo-Mediterranean	5353	AWG	3	ARG	36	15	13	3	---	---	---	---	×120	365	4	1.1
				2	AWV	---	16	---	---	---	45	---	---	×130			
				2	BWG	---	---	---	34	---	---	---	---	(c)			
				1	ARG	22	---	4	---	---	---	---	---	×121 or 129			
120	Goens	5354	BRG	3	ARG	40	15	12	4	---	---	---	---	×119	361	11	3.0
				1	ARV	8	---	3	---	13	---	5	---	[d]×130			
				1	AWG	---	22	---	---	---	---	---	---	M119			
131	Mediterranean	5364	BRG	3	ARV	8	14	6	2	37	12	14	7	×130	142	7	1.7
				7	BRG	---	---	135	49	---	---	---	---	×131			
				5	AWG	---	08	---	25	---	---	---	---	×118			
132	Stoner	5365	BWG											or 134	176	60	34.1
				15	ARG	200	74	77	25	---	---	---	---	×365			
				10	AWV	---	61	---	19	---	201	---	65	×130			
				11	ARG	152	51	48	16	---	---	---	---	×110 or 138			
139	Red Wonder	5373	BWG											or 358	200	14	7.0
				3	AWG	---	34	---	14	---	---	---	---	×137 or 359			
				6	ARG	86	31	29	12	---	---	---	---	×94			
				3	AWG	---	54	---	30	---	---	---	---	×154			
155	Fulcaster	5389	BWG	2	ARV	9	5	1	1	23	8	10	4	×342	192	20	10.4
				1	BRV	---	---	6	0	---	---	14	10	×157			
				1	BRG	---	---	9	6	---	---	---	---	× ?			

[a] A=awnless, B=bearded or awned, R=red chaffed, W=white chaffed, V=velvet or pubescent, G=glabrous.

[b] ×=result of natural cross with row given, M=result of mechanical mixture with row given.

[c] Probably mechanical mixture or possibly segregates of natural cross previous to 1917.

[d] Segregation for chaff color, although evident, could not be accurately determined on account of discoloration of glumes.

TABLE 1.—*Varieties of wheat grown in 18-foot rows at Arlington Experiment Farm in 1918 in which off-type plants were found, with head characters of the varieties and off-type plants, the probable origin of the off-type plants based on their breeding behavior, and the percentage of natural F_1 hybrids from 1917 seed*—Continued

Row No. in 1918	Variety	C. I. No.	Head characters of variety	Off-type plants in 1918 rows of the variety: Number	Off-type plants in 1918 rows of the variety: Head characters	Breeding behavior of off-type plants in 1919 (number of plants with given head characters): ARG	AWG	BRG	BWG	ARV	AWV	BRV	BWV	Probable origin of the off-type plants	Number of plants grown in 1919 from 1917 seed remnants: Total	Number with head characters like F_1 hybrids in 1918 rows	Percentage of F_1 hybrids from 1917 seed
159	Stoner	5394	BWG	6	BRV			35				88		[d] ×157			
				5	ARV	18		10		78		24		[d] ×158			
				14	ARG	167	55	56	21					×90 or 160	158	43	27.2
				7	AWG		116		35					×161			
				2	ARG	30								M90 or 160			
515	Rudy	3512	BWG	6	ARG	92	41	28	11					×514			
				1	AWG		17		5					×516	140	4	2.8
				2	AWV		9		3		35		14	×726			
				7	ARG	86	33	30	7					×550 or 552			
551	Rudy (Queen of New York)	3493	BWG	2	AWG		42		12					×443 or 688	238	5	2.1
				1	ARG	18								M550 or 552			
568	Penquite	3539	BRV	2	ARV					36		14					
				2	ARV					31	8	11	2				
				3	ARV					71	25						
				2	AWV						29		14	[e]	237	[e]20	[e]8.4
				1	BRV							23	7				
				2	BWV								52				
				2	AWV						52						
576	Prosperity	3558	AWG	3	AWV		14		13		60		18	×575			
				3	ARG	41	13	19	6					×665	221	6	2.7
				4	ARG	98	31							×417 or 577			
583	No. 8	3570	AWG	1	ARG	24	9							×582			
				1	ARG	18	6	6	1					×660	247	2	.8
594	(Selection)	----	BRG	2	ARG	48		18						×593	----	----	----
599	White Chaff	359	AWG	1	AWV		4				26			×600 or 642			
				2	ARG	39	13							×598 or 394			
				1	ARG	[d] 21		[d] 7						× ?	----	----	----
				4	AWV						122			M600 or 642			
701	Fulcaster	3406	BWG	1	AWV		3		1		20		7	× ?			
				4	BRG			59	20					×703			
				12	AWG		226		63					×540	----	----	----
				22	ARG	464	180	140	47					×702 or 700 or 788			
702	Fultzo-Mediterranean	3418	AWG	11	ARG	223	75							×700 or 788	237	15	6.3
710	Millers Pride	347	0 BRG	5	ARV	25		9		76		23		[d] ×709			
				10	AWG		227							M711	154	2	1.3
711	New Monarch	3477	AWG	3	AWV		18				49			×709			
				3	ARG	38	15	13	5					×710 or 778			
				5	ARG	90	29							×531 or 529	233	11	4.7
712	Pride of Genesee	3491	BWV	1	AWG		15		7					×530			
				2	ARV	14		5			43		12	×713 or 529	150	0	----

[d] Segregation for chaff color, although evident, could not be accurately determined on account of discoloration of glumes.

[e] The off-type plants in this variety are possibly the segregates of a cross between the variety Fishhead (AWV), which was grown next to Penquite (BRV) in 1916. The ARV type may have been overlooked in roguing the 1917 seed heads.

In order to obtain accurate data on the percentage of natural crossing that had taken place in 1917, from 240 to 600 kernels from the 1917 seed remnants of each of 16 of the strains showing off-type plants in 1918 also were space planted in the fall of 1918. From 50 to 80 per cent of these produced plants in 1919, and at harvest time descriptive head data on individual plants were obtained. The off types present were compared with those present in the 1918 rod rows. From the breeding behavior of the 1918 off types, as found in 1919, and the result of growing spaced plants from the 1917 remnant seed, the percentage of F_1 hybrid plants produced from the 1917 seed has been calculated, as shown in the last column of Table 1. The off-type plants present in the 1919 rows from 1917 seed remnants were not tested further, but it is assumed that their breeding behavior would have been the same as that of the off-type plants from the 1918 crop tested in 1919.

A total of 248 off-type plants of the crop of 1918 were tested by growing a head from each in 1919. Of these, 214 segregated in such a manner as to indicate that they were F_1 hybrids. Of the remaining 34 plants, 20 were evidently mechanical mixtures from adjoining rows. Ten of these were in row 710 and were apparently mixtures with row 711. The remaining 14 plants, occurring in row 568 (Penquite), have been interpreted as due to a cross occurring previous to 1917, of which the F_1, or a heterozygous segregate, was inadvertently included among the seed heads collected from the 1917 crop. Such a cross may have occurred in 1916 when the variety Fishhead, which is awnless with white velvet chaff, was grown next to Penquite, which is bearded with red velvet chaff. The F_1 of this cross is semiawned and red chaffed, and a head of this kind may have been overlooked in the 1917 seed heads.

In several cases noted in Table 1, especially in wheats with velvet or pubescent chaff, the color of the chaff was difficult or sometimes impossible of determination as a result of poor development of color, or of discoloration due to weathering or fungous growths.

The number of hybrid plants present in the 1918 rows of the five strains of the Fulcaster type grown in rows 132 (Stoner), 139 (Red Wonder), 155 (Fulcaster), 159 (Stoner), and 701 (Fulcaster) is especially noteworthy. The percentage of probable F_1 hybrids present in the plants from the 1917 seed remnants in four of these strains tested in 1919 ranged from 7 to 34.1 per cent, being higher than in all other varieties with the possible exception of Penquite (row 568). There is no doubt that unusually heavy natural crossing occurred in the strains of the Fulcaster type in 1917, especially in rows 132 and 159, in which the percentages of hybrid plants were 34.1 and 27.2 per cent, respectively. Three recessive characters are combined in the Fulcaster type of wheat—presence of awns, white glumes, and glabrous glumes. Natural crosses with awnless, red-glumed, or velvet-glumed wheats are readily recognized.

All of the 214 segregating off-type plants tested in 1919 behaved as F_1 hybrids, segregation occurring in approximately the expected ratios. If crossing had occurred previous to 1917, segregates of later generations than the F_1 would have been present and some individuals of several groups with different head characters should be homozygous. Such homozygous forms were not present, so it

appears that all of these 214 off types were actually F_1. It is also evident that the true-breeding plants interpreted as mechanical mixtures were not homozygous segregates of earlier natural crosses. An instance may be cited. Two awnless-red-glabrous (ARG) glumed plants in Stoner, row 159, bred true to head type. These are interpreted as mechanical mixtures with the adjacent row 160 or with row 90 directly across the alley, inasmuch as in a cross of a BWG × ARG occurring previous to 1917 it would be expected that true-breeding BRG and AWG forms, as well as ARG and BWG, would be present in the 1918 row. Likewise 3 of the 5 segregating genotypes expected in such a cross were not present, while the 2 that were present are more probably due to natural crossing with neighboring rows in 1917.

It is concluded, then, that the segregating off-type plants were F_1 hybrids and that the true breeding off types are due to mechanical mixtures, with the exceptions noted.

The above-stated conclusions are strengthened by the fact that it has been possible to determine the probable origin of the off-type plants, as shown in Table 1. In nearly every case the hybrid type found would have resulted if crossing had taken place with plants in adjoining rows or in rows not more than 2 or 3 feet away. The characters possessed by the plants in different rows from which off-type selections were made and by those in their neighboring rows, are shown in Figure 1. Nearly every case of natural crossing has been explained as due to cross-pollinations that may have occurred between plants so located that they may have come into actual physical contact with each other at the time of blooming. A few exceptions are found where the probable parents can not be determined. These may have resulted from transportation of pollen from greater distances, but possibly are due to crosses with mixtures in the row or to other undetermined causes.

The wheat crop of 1917 produced the highest average grain yield in any year of the period 1910 to 1925, inclusive. The precipitation during the spring months was below the average, but during the period from May 15 to 31, inclusive, when most of the blooming occurred, the rainfall totaled 1.06 inches.

The season of 1917, though unusually favorable to the general run of wheat varieties, was comparatively unfavorable to the Fulcaster strains. The average yield of the 22 varieties or strains grown in the varietal plot test was 46.2 bushels per acre, while the average yield of the five Fulcaster types was only 37.2 bushels, the different strains ranging in yield from 34.4 to 38.9 bushels. The three Poole selections averaged 51 bushels, the two Purplestraws 49.9 bushels, and the four Mediterraneans 46.4 bushels. In the rod-row nursery the yield of the Fulcasters was comparable. It is probable that the factors resulting in the comparatively poorer yielding performance of the Fulcasters in 1917 were also factors having to do with natural crossing, as no doubt this type did not produce as much pollen with which to pollinate its own flowers as the Pooles or Purplestraws.

The method of selecting seed heads in 1917 may have resulted in a greater percentage of hybrid plants being found in 1918 than if the seed had been taken from the entire row. In selecting heads for seed purposes in 1917 it was necessary in many cases to take those from secondary tillers. The results obtained in 1924 show that a much

greater percentage of natural crossing occurs in the later secondary tillers than in the earlier primary ones. As these seed plants grew at the ends of the rows, the number of secondary tillers would be greater in proportion than occurs on plants not at the ends. It would happen, then, that more secondary heads would be included in the seed lots than the average for the row.

NATURAL HYBRIDS IN THE 1921 FIELD PLOTS

The wheat varieties tested at Arlington Experiment Farm for yield in the fortieth-acre plots contain each year many off-type plants which are of hybrid origin. In the crop of 1921 the variety Nebraska No. 28, which is bearded, with white glabrous glumes and yellow straw, contained about 13 per cent of off-type heads, which were semiawned with glabrous white chaff and purple straw. Natural crossing with a wheat of the Purplestraw or Fultz type was thereby indicated, as the 1920 seed plot of Nebraska No. 28 had been carefully rogued by both writers. The 1921 plots of the variety Kanred contained off types of several kinds, but the total percentage of these was lower than in Nebraska No. 28.

Heads were harvested from representatives of the off types present in 1921 in the four varieties Nebraska No. 28, Kanred, Purplestraw, and Bearded Purplestraw. Each head represented one plant, and the seed of these was space-planted in head rows in the fall of 1921. Poor germination and drowning greatly reduced the stand in many rows, particularly in the selections from Nebraska No. 28, of which 4 of the 15 rows contained 4 or less plants each, none being fully bearded. It is probable, however, that the selections were all alike in constitution, so all of them are combined in the data on breeding behavior given in Table 2. Of 71 plants grown from off types selected from Nebraska No. 28, 51 were awnless (or semiawned) and 20 bearded, which is very near the ratio expected in the F_2 of a cross between an awnless and a bearded wheat. It appears that all 15 selections in Nebraska No. 28 were natural hybrids. It is certain that Nebraska No. 28 was largely cross-fertilized in 1920 by an awnless wheat with purple straw, probably the variety Purplestraw, which begins to bloom a few days later than Nebraska No. 28 but before the latter has finished blooming.

From the 53 off-type heads selected in Kanred, a bearded-white-glabrous-chaffed wheat, 22 proved to be hybrids. The types selected were varied in head character. The bearded-white-glabrous types were selected because they showed greater vigor than Kanred and did not have the Kanred beak. Some of these may have been hybrid, but the differentiating characters were not sufficiently pronounced to make it certain. In the progeny of four of these bearded heads, supposedly recessive in type, awnless forms appeared, indicating that natural crossing had occurred in these heads in 1921.

Six of the 27 selections in Purplestraw and 2 of the 6 in Bearded Purplestraw segregated in such a way as to indicate that they were F_1 hybrids. Ten awnless-white-glabrous heads selected in Purplestraw bred true and probably were merely fluctuating variations in the variety. Other pure-breeding selections in Kanred, Purplestraw, and Bearded Purplestraw were probably mechanical mixtures, or possibly in some instances homozygous segregates of crosses occurring previous to 1920.

TABLE 2.—*Numbers and characters of off-type heads selected in four varieties of winter wheat at Arlington Experiment Farm in 1921, with their breeding behavior in 1922*

Variety	C. I. No.	Head characters of variety[a]	Off-type plants in 1921		Breeding behavior of off-type plants in 1922 (number of plants with given head characters)[a]					
			Number	Head characters[a]	AWG	BWG	AWV	BWV	ARG	BRG
Nebraska No. 28	5147	BWG	15	AWG	51	20				
Kanred	5146	BWG	17	AWG	188	66				
			2	AWV	9	1	22	8		
			1	BRG		4				4
			1	AWV			16	2		
			1	ARG					17	5
			16	BWG		Pure.				
			6	AWG	Pure.					
			2	BRG						Pure.
			1	ARG					Pure.	
			2	AWV			Pure.			
			1	BWG	[b]3	16				
			1	BWG	[b]7	10				
			1	BWG	[b]1	5				
			1	BWG	[b]1	14				
Purplestraw	1915	AWG	4	AWG	38	10				
			2	ARG	5				33	
			10	AWG	Pure.					
			3	BWG		Pure.				
			3	AWV			Pure.			
			5	ARG					Pure.	
Bearded Purplestraw	1911	BWG	2	AWG	22	6				
			4	AWG	Pure.					

[a] A=awnless, B=bearded or awned, G=glabrous chaffed, R=red chaffed, W=white chaffed, V=velvet or pubescent chaffed.
[b] Behavior due to natural crossing in 1921.

NATURAL CROSSING IN 1921

In addition to the plant selections made in 1921, an experiment was conducted in which the varieties Gladden, Grandprize, Kanred, Brown Fife, and Fulcaster were grown in the order given in single rows 132 feet long and 8 inches apart. Grandprize and Brown Fife are awnless with red pubescent chaff; all the other varieties are bearded with white glabrous chaff. The seedings were made in the fall of 1920, and at harvest in 1921 from 40 to 50 heads representative of each of the varieties except Gladden were selected and hand threshed. A portion of this seed was sown in spaced rows in the fall of 1921. The 1922 crop from this seed showed no off types in any variety except Kanred, which contained 11, or 2.64 per cent, natural hybrids in the 416 plants matured. It is possible, however, that all of the other three varieties may have contained natural hybrids not evident in F_1. Descriptions of the off types in Kanred and their F_2 segregation in 1923 are given in Table 3.

In another experiment with Kanred, 25 heads typical of the variety were selected from a plot in 1921. From the seed of these heads 221 plants were grown in 1922, among which 6 F_1 hybrids, or 2.71 per cent, were found. The F_2 segregation in 1923 of these 6 plants is shown in Table 3. Practically the same percentage of natural crossing occurred in the field plot of the Kanred variety in 1921 as occurred in the row specially planted for determination of the extent of natural crossing in contiguous rows.

TABLE 3.—*Head characters and breeding behavior of natural F_1 hybrids developed from seed of typical heads of Kanred wheat grown in a specially planted row and of natural F_1 hybrids developed from typical heads of Kanred grown in a plot at Arlington Experiment Farm*

F_1 natural hybrids in 1922			F_2 segregation in 1923 (number of plants with given head characters) [a]							
Source and number	Per cent	Head characters [a]	AWG	BWG	ARG	BRG	ARV	BRV	AWV	BWV
From rows:										
7	1.68	ARV	29	8	50	13	117	50	43	20
4	.96	AWG	76	30						
Total	2.64									
From a plot:										
1	.45	ARG	11	2	12	9				
5	2.26	AWG	64	19						
Total	2.71									

[a] A=awnless, B=bearded or awned, R=red chaffed, W=white chaffed, G=glabrous chaffed, V=velvet or pubescent chaffed.

EXPERIMENTS IN 1924 TO 1926

Six varieties of wheat, two varieties of spelt, one variety of emmer, and *Aegilops ovata* were sown on October 15, 1923, in 52-foot rows 8 inches apart, in the order shown in Table 4. The varieties were chosen and the order of seeding determined with reference to the head characters and the time of blooming of the varieties, as shown in Table 4. Abruzzes rye was sown in 40-foot rows in a block adjoining one end of the rows of these varieties, and Rosen rye was sown similarly in a block adjoining the other end. This experimental plot was isolated from other sowings, except four plots of Alstroum spelt separated from it by a fortieth-acre plot of winter oats. This seeding of spelt was made October 8 and apparently had no effect on the results obtained.

TABLE 4.—*Head characters, blooming period, and seeding arrangement of varieties of grains grown at Arlington Experiment Farm in 1924 for studies of natural crossing*

Block 1, 40-foot rows	Block 2, 52-foot rows			Block 3, 40-foot rows
Rye variety	Varieties of wheat and other grains	Head characters [a]	Blooming period in 1924	Rye variety
Abruzzes (12 rows)	1. Purplestraw	AWG	May 26–June 2	Rosen (12 rows)
	2. Nebraska No. 28	BWG	May 29–June 2	
	3. Red Rock	BRG	May 31–June 6	
	4. Dawson	ARG	June 1–7	
	5. Brown Fife	ARV	ditto	
	6. Kanred	BWG	ditto	
	7. Alstroum spelt	AWG	June 2–9	
	8. White Bearded spelt	BWG	June 2–8	
	9. Black Winter emmer	BBlV	June 8–12	
	10. Nebraska No. 28	BWG	May 22–June 2	
	11. Purplestraw	AWG	May 26–June 2	
	12. Aegilops ovata	BWS	May 26–June 3	

[a] A=awnless, B=bearded or awned, G=glabrous chaffed, R=red chaffed, S=glumes covered with short spines, V=velvet or pubescent chaffed, W=white chaffed, Bl=black chaffed.

At heading time the rows were carefully examined for off-type plants, which were removed before flowering. To insure selfing 50 heads of Nebraska No. 28, representing as many plants, were bagged before flowering. This variety originated as a selection from a cross of Big Frame and Turkey, and it was thought that perhaps some of the off-type plants often found in it might be occasional segregates of the original cross. At harvest time in 1924, heads were selected from each wheat variety or species and from *Aegilops ovata*. No material was selected from rows 10 and 11, which were duplicates of rows 2 and 1. In the spelts, Black Winter emmer, Kanred, and Aegilops, approximately 40 heads were taken at random, but in the remaining five common wheats a first selection of about 40 heads from each variety included only large heads from tall, vigorous, or primary culms, while a second selection of about 40 heads each was confined to shorter, less vigorous, or secondary culms. These two lots are designated "primary" and "secondary," respectively. All heads were carefully examined for trueness to variety or type and then hand threshed. In the fall of 1924 several hundred kernels of each lot were sown in rows with 4-inch spacing between kernels, but drowning of plants reduced the stand in several instances. It was evident from the plants produced from this seed that considerable natural crossing had occurred in 1924 in most of the wheat varieties, though no natural hybrids between wheat and rye were found. Stand notes were taken, and a head from each recognizable off-type plant present in the variety or species was harvested. All off types, except 12 in Kanred, which were unmistakably hybrid, were hand threshed and grown in spaced rows for segregation data.

In those varieties or species in which dominant head characters could easily obscure an F_1 hybrid, all plants showing such characters as increased height or vigor, lighter glume color, or variation in awning as compared with the type of the variety or species were tested by growing seed from a head of the possible off-type plant. Many of these, as shown by later tests, were merely fluctuating variations. In Dawson, 5 from a total of 42 plants, possessing head characters similar to Dawson but differing in vigor or with lighter red glume color, proved to be hybrid. In Brown Fife, 2 of the 49 possible off types with characters similar to Brown Fife were natural hybrids.

The data on segregation given in Table 5 show that all off-type plants possessing a dominant character, such as absence of beards, present in a variety characterized by its recessive allelomorph, such as presence of beards, were F_1 hybrids.

The head characters of the varieties used in the experiment permitted recognition of the pollen parent of the natural cross. One exception occurred in an awnless, red-glumed plant present in Dawson, described in 1925 as a speltoid type. It was distinctly less spelt-like than the natural hybrids which occurred frequently between the spelts and common wheats. This speltoid plant in 1926 produced nine plants classed as speltoid and nine classed as wheat. All 18 of these were red glumed and white kerneled, indicating that either natural crossing between Dawson strains carrying factors for the spelt form or mutation was responsible for this type of inheritance. The Dawson variety was selected for purity to awnlessness, red glumes, and white seeds, and is not necessarily pure for other characters.

TABLE 5.—*Varieties or species of Triticum and Aegilops grown in an isolated plot at Arlington Experiment Farm in 1924 from which plants were selected in 1925 for testing, with head characters of the variety, species, and selected plants, and the pollen parent of hybrid forms, based on the breeding behavior in 1926 of the selected plants*

Variety and species	C. I. No.	Head characters of variety [a]	Plants selected for testing		Breeding behavior of selected plants (number of plants with given head characters) [a]															Pollen parent of hybrid forms
			Number	Head characters [a]	Spelt types								Wheat types							
					ARV	ARG	BRV	BRG	AWV	BWV	AWG	BWG	ARV	ARG	BRV	BRG	AWV	BWV	AWG	BWG
Nebraska No. 28 (Triticum vulgare)	5147	BWG	62	AWG															1,637	509	Purplestraw.
			9	ARV									129	45	48	19	55	20	19	4	Brown Fife.
			14	BRG												322				102	Red Rock.
			8	AWG [b]							164	47							40	23	Alstroum.
			6	BWG [b]								123								48	White Bearded.
			3	ARG										46		18			21	6	Dawson.
			1	BWG [c]																27	Kanred.
Purplestraw (T. vulgare)	1915	AWG	15	ARG										193		85			79	25	Red Rock.
			3	ARG										66					16		Dawson.
			7	ARV									143	44			48		21		Brown Fife.
			4	AWG [b]							58	30							16	1	White Bearded
			4	AWG [b]							81								28		Alstroum.
			2	AWG [d]															63		
Red Rock (T. vulgare)	5976	BRG	2	ARG										44		24					Dawson.
			3	ARG										52		15			13	4	Purplestraw.
			1	ARV									21	6	6	0					Brown Fife.
			1	ARG [b]		14		2			5	1		2		1			0	0	Alstroum.
			14	BRG [d]												402					
Dawson (T. vulgare)	6161	ARG	6	ARG [b]		73		23			26	6		28		1			18	2	White Bearded.
			5	ARG [b]		82					26			19					11		Alstroum.
			1	ARG [e]		9								9							?
			5	ARG										95					28		Purplestraw.
			2	ARV									45	20							Brown Fife.
			37	ARG [d]										844							

[a] A=awnless or half awned, B=bearded or awned, G=glabrous chaffed, R=red chaffed, S=glumes covered with short spines, V=velvet or pubescent chaffed, W=white chaffed, Bl=black chaffed.

[b] Typical hybrid type of *Triticum vulgare* × *T. spelta*.

[c] Segregating for Kanred beak character.

[d] Homozygous selections.

[e] Speltoid type.

TABLE 5.—*Varieties or species of Triticum and Aegilops grown in an isolated plot at Arlington Experiment Farm in 1924 from which plants were selected in 1925 for testing, with head characters of the variety, species, and selected plants, and the pollen parent of hybrid forms, based on the breeding behavior in 1926 of the selected plants*—Continued

Variety and species	C. I. No.	Head characters of variety	Plants selected for testing		Breeding behavior of selected plants (number of plants with given head characters)														Pollen parent of hybrid forms
					Spelt types								Wheat types						
			Number	Head characters	ARV	ARG	BRV	BRG	AWV	BWV	AWG	BWG	ARV	ARG	BRV	BRG	AWV	BWV	AWG	BWG	
Brown Fife (T. vulgare)	1933	ARV	8	ARV [b]	78	24	23	6	25	9	11	2	29	14	4	0	8	0	3	0	White Bearded.
			8	ARV [b]	94	38			34	7			31	13			10		5		Alstroum.
			1	ARV									10	7	8	3					Red Rock.
			1	ARV									17	5	4	3	4	3	3	0	Kanred.
			47	ARV [d]									1,504								
Kanred (T. vulgare)	5146	BWG	8	ARV									47	15	11	5	15	4	3	2	Brown Fife.
			6	BRG												60				23	Red Rock.
			4	BWG [b]								51								19	White Bearded.
			4	AWG [b]							43	8							10	10	Alstroum.
			2	ARG										5		4			4	1	Dawson.
			5	BWG [d]																124	
White Bearded (T. spelta).	1724	BWG	2	ARV [b]	11	3	7	4	3	7	1	2	1	2	0	0	3	0	0	0	Brown Fife.
			3	BWG [d]								69									
Alstroum (T. spelta)	1773	AWG	12	AWG [d]							268										
Black Winter (T. dicoccum).	2337	B Bl V	0																		
Ægilops ovata		BWS	2	AWS																	Purplestraw.

[b] Typical hybrid type of *Triticum vulgare* × *T. spelta*.

[d] Homozygous selections.

Purplestraw was the pollen parent of 72 of the natural hybrids listed in Table 5, of which 62 were present in the Nebraska No. 28 variety. Red Rock was the pollen parent in 36 hybrids, Alstroum spelt in 30, Brown Fife in 29, White Bearded spelt in 28, Dawson in 10, and Kanred in 2. Nebraska No. 28, Black Winter emmer, and *Aegilops ovata* did not cross-fertilize any of the varieties or species in 1924. Black Winter emmer and Alstroum spelt were entirely self-fertilized. Seed of *A. ovata* produced two natural hybrids, the result of cross-pollination with Purplestraw, both of which were sterile. White Bearded spelt was self-fertilized with the exception of two natural hybrids with Brown Fife. All six common wheat varieties were cross-fertilized by at least four different pollen parents. Nebraska No. 28 was the pistillate parent to seven different groups of natural hybrids. Only Black Winter emmer and *A. ovata* failed to pollinate Nebraska No. 28.

In Table 6 is shown the frequency of crossing between the varieties of wheat or spelt, expressed as percentages of the plants tested for natural hybrids in 1925, as listed in Tables 5 and 7. From the types of segregates appearing in F_2 it was possible to determine the parentage of the hybrid forms, the parents being restricted to those grown together in the isolated plot in 1924. The tested plants from each variety that were not hybrid were obviously due to selfing within the variety. The percentage of selfing in each variety also appears in Table 6. The high receptivity of Nebraska No. 28 and Kanred to wind-borne pollen and the infrequency of their action as pollinizers are apparent from these results.

TABLE 6.—*Percentage frequency of wheat and spelt varieties as reciprocal male and female parents of the natural hybrids listed in Table 5, and the percentage of self-fertilization in each variety*

Female parent	Male parent							
	Nebraska No. 28	Purple-straw	Red Rock	Dawson	Brown Fife	Kanred	White Bearded spelt	Alstroum spelt
Nebraska No. 28	78.4	13.0	2.9	0.6	1.9	0.2	1.3	1.7
Purplestraw	0	97.6	1.1	.2	.5	0	.3	.3
Red Rock	0	.4	99.2	.2	.1	0	0	.1
Dawson	0	.7	0	97.5	.3	0	.8	.7
Brown Fife	0	0	.1	0	97.8	.1	1.0	1.0
Kanred	0	0	2.2	.7	3.0	91.1	1.5	1.5
White Bearded spelt	0	0	0	0	.5	0	99.5	0
Alstroum spelt	0	0	0	0	0	0	0	100

The seeding arrangement of the several forms of wheat, spelt, emmer, and Aegilops included in the original isolated plots in the fall of 1923 was determined partly by the time of blooming of the different forms. From the data given in Table 4 it is seen that blooming was continuous from May 22 to June 12 and that the forms in adjoining rows, with the possible exception of Black Winter emmer, coincided more or less in their time of blooming. Presumably, therefore, abundant opportunity for cross-pollination occurred, except in Black Winter emmer, in which no hybrid plants were found.

TABLE 7.—*Numbers of plants grown and numbers and percentage of natural F_1 hybrids, as listed in Table 5, produced by seed from primary and secondary heads or heads taken at random in 1924*

Variety	C. I. No.	Number of plants grown from seed of—			Number of natural F_1 hybrids in plants from seed of—			Percentage of natural F_1 hybrids in plants from seed of—		
		Random heads	Primary heads	Secondary heads	Random heads	Primary heads	Secondary heads	Random heads	Primary heads	Secondary heads
Purplestraw	1915		804	562		8	25		1.0	4.4
Nebraska No. 28	5147		144	332		15	88		10.4	26.5
Do.*	5147	315			0			0		
Red Rock	5976		553	267		3	4		.5	1.5
Dawson	6161		520	220		7	12		1.3	5.5
Brown Fife	1933		520	286		4	14		.8	4.9
Kanred	5146	267			24			9.0		
Alstroum spelt	1733	439			0			0		
White Bearded spelt	1724	382			2			.5		
Black Winter emmer	2337	306			0			0		
Aegilops ovata		189			2			1.1		
Total percentage of natural crossing									1.5	8.6

* Seed from bagged heads.

Crossing was general among the varieties included in this seeding, occurring frequently between varieties separated by several rows as well as between varieties growing side by side. Four crosses were recorded between Purplestraw wheat and Alstroum spelt grown 32 inches apart in rows 11 and 7. From other observations it is known, however, that wheat pollen may be transported and effect cross-fertilization from considerably greater distances than this.

In the harvest of 1924, heads were taken from primary and secondary culms of five varieties of wheat in the isolated plot, but were taken only at random from the other sowings. The numbers of plants grown in 1925 from seed of these different head lots are shown in Table 7, together with the number and percentage of natural F_1 hybrids included in these plants in each lot. The hybrid nature of the plants was determined by their segregation in 1926, as shown in Table 5. The percentages of F_1 hybrids in the plants from seed of secondary heads were from approximately two to six times as large as the percentages in the plants from primary heads. The percentage of natural hybrids in all plants from seed of the primary heads of the five varieties was 1.5 per cent; in all plants from seed of the secondary heads it was 8.6 per cent, or about six times larger than from the primary ones.

More than one-fifth of the plants of Nebraska No. 28 were F_1 hybrids when grown from seed of open-fertilized heads. Among the 315 plants of this variety, however, grown from seed of bagged and therefore self-fertilized heads, no hybrid plant occurred. This indicates that the variety is pure breeding and that the off types that usually occur in it in such large numbers are due to natural crossing and are not occasional segregates of the original cross.

DISCUSSION

In these studies on natural hybridization in wheat, during the 10-year period from 1917 to 1926, the percentage of hybrids found in the different varieties under observation varies from zero to

approximately 34 per cent. Although these investigations were designed to establish the fact and the extent of natural crossing rather than the causes, certain indications as to the factors that are responsible for its occurrence have been obtained.

It appears that seasonal conditions play an important rôle in the extent of natural crossing that occurs, but that in these conditions several factors are concerned. Among these factors rainfall and temperature are probably important. In order to study the relation of rainfall and temperature to natural crossing, Table 8 was prepared. In it are given the daily precipitation in inches and the maximum and minimum temperatures in degrees Fahrenheit from May 1 to June 9 in the years 1917, 1920, 1921, and 1924, and the blooming periods of the varieties and species of wheat studied each year. The same varieties were not under observation throughout the entire period, and those that were included for more than one year were grown under somewhat different conditions from year to year.

Extensive natural crossing occurred in 1917, a year when spring rainfall (April, May, and June) was 1.5 inches below the 15-year average (1912–1926), though, as may be seen in Table 8, there were several showers during the latter part of the blooming period. Only 0.15 inch of rain fell in three showers from May 5 to May 26; blooming began May 18. In 1920 the May rainfall was only 1.6 inches as compared with the 15-year average of 3.51 inches, and no rain fell during the blooming period of the varieties observed. In this year about 13 per cent of natural crossing occurred in Nebraska No. 28. In 1924 Nebraska No. 28 bloomed during a period of frequent showers, rain falling on 7 of the 11 days of the blooming period and totaling 2.31 inches. In this year 21.6 per cent natural crossing occurred in a row of Nebraska No. 28 grown near other varieties. In 1921 Kanred was cross-fertilized by near-by varieties to the extent of approximately 2.7 per cent, as shown by the natural hybrids in 1922; in 1924 the cross-fertilization in Kanred amounted to 9.0 per cent. In 1921 the spring rainfall was 2.65 inches above the 15-year average and the May rainfall 2.95 inches above the 15-year average for the month; rainfall totaling 0.26 inch occurred, however, only on the first and last days of the blooming period of the Kanred variety. In 1924 the rainfall in April, May, and June was 15.21 inches, or 4.21 inches above the average, the highest recorded at Arlington in the 15-year period; and the May rainfall of 6.59 inches, or 3.08 inches above average, surpassed all previous years of this period. Kanred bloomed during a comparatively rainless period, rain occurring only on the first and last days and totaling 0.61 of an inch.

Although showers occurred frequently in 1924 during the blooming period of Nebraska No. 28 and infrequently during the blooming period of Kanred, unusually extensive natural crossing took place in both varieties. In 1920, when Nebraska No. 28 bloomed during a rainless period, 13 per cent natural crossing occurred in that variety, while in the rainy period of 1924, 21.6 per cent natural crossing occurred. The relation of rainfall to natural crossing is not apparent from the behavior observed in these and also in other varieties.

76728—27——2

TABLE 8.—*Daily precipitation (in inches) and maximum and minimum temperatures (in degrees F.) from May 1 to June 9, inclusive, for the years 1917, 1920, 1921, and 1924, at Arlington Experiment Farm*

[The limits of the blooming period of the varieties of winter wheat under observation in which natural hybrids occurred are shown by parallel dashes across the three columns pertaining to each year]

Date	1917			1920			1921			1924		
	Precipitation	Temperature		Precipitation	Temperature		Precipitation	Temperature		Precipitation	Temperature	
		Maximum	Minimum		Maximum	Minimum		Maximum	Minimum		Maximum	Minimum
	Inches	*° F.*	*° F.*	*Inches*	*° F.*	*° F.*	*Inches*	*° F.*	*° F.*	*Inches*	*° F.*	*° F.*
May 1	0.22	81	55	0.38	64	47	1.31	52	48	-------	68	53
2	-------	68	45	-------	63	34	-------	62	45	-------	74	39
3	-------	65	46	-------	64	38	-------	57	46	-------	79	44
4	.87	50	44	-------	65	39	.91	52	47	-------	68	43
5	-------	47	44	-------	65	42	.53	50	48	-------	75	37
6	.01	56	43	-------	68	34	.10	58	46	-------	92	47
7	-------	59	43	-------	62	45	-------	74	43	-------	78	59
8	.05	52	47	.41	67	52	-------	75	49	1.23	69	60
9	-------	62	44	-------	72	42	-------	78	51	.40	70	56
10	-------	64	39	-------	82	44	-------	83	47	.02	64	54
11	-------	70	39	-------	85	48	-------	61	56	-------	54	51
12	-------	63	46	-------	58	54	1.90	60	51	1.57	62	48
13	-------	66	47	.76	52	50	.34	78	51	.10	76	52
14	-------	73	49	-------	58	49	.05	76	58	-------	75	52
15	-------	74	53	.05	64	41	-------	78	59	.50	67	47
16	-------	81	43	-------	70	35	.25	63	53	.34	66	51
17	-------	81	56	-------	70	38	-------	74	44	-------	74	45
18	-------	87	48	-------	74	40	-------	79	45	.04	79	53
19	-------	90	55	-------	73	52	-------	80	52	-------	76	53
20	-------	91	55	-------	78	53	-------	80	50	-------	65	57
21	-------	74	61	-------	78	60	-------	85	55	.66	57	51
22	-------	81	57	-------	81	58	-------	89	60	.16	65	48
23	.09	72	62	-------	69	53	-------	90	62	-------	80	43
24	-------	74	45	-------	59	56	.01	61	53	-------	75	63
25	-------	65	46	-------	67	54	.56	75	49	.64	66	47
26	-------	74	45	-------	72	56	-------	76	55	.14	70	45
27	.39	90	58	-------	80	46	-------	71	53	.58	62	56
28	.21	66	60	-------	83	52	-------	85	58	.04	78	58
29	.37	69	57	-------	84	56	-------	83	63	-------	73	57
30	-------	81	49	-------	84	51	.50	76	66	.17	69	53
31	-------	76	55	-------	81	47	-------	82	55	-------	72	44
June 1	-------	86	61	-------	87	52	-------	85	53	.58	75	48
2	.04	86	62	-------	90	61	-------	78	62	-------	75	54
3	.34	85	64	-------	88	65	-------	82	52	-------	74	56
4	-------	82	55	-------	60	58	-------	82	61	-------	75	60
5	-------	83	57	1.19	61	55	-------	78	52	-------	78	59
6	.11	85	67	.83	70	52	-------	79	49	-------	86	55
7	1.57	81	65	-------	78	52	-------	72	59	.03	86	60
8	-------	85	60	-------	80	51	-------	80	60	-------	85	63
9	-------	84	60	-------	76	55	-------	82	65	1.48	66	57

When temperature is considered, the relationship with natural crossing again is not apparent. The extreme diurnal temperature ranges (in degrees Fahrenheit) for the blooming periods were 45–91 in 1917, 35–90 in 1920, 44–90 in 1921, and 43–86 in 1924. It is known that very little blooming occurs when the temperature is below 60°, and that usually none at all occurs at temperatures below about 56°. It is not known, of course, when the flowers bloomed which were cross-fertilized; consequently, the temperatures at which crossing occurs or the temperature conditions during the blooming period or preceding it that favor crossing can only be surmised. It is known, however, that during periods of low temperatures and during rainy

periods, possibly but not certainly when temperature is high as well as low, blooming is delayed until favorable weather again occurs, when dehiscence becomes general. Under these conditions, varieties normally blooming several days apart will open and shed their pollen simultaneously, thus allowing natural crossing to occur between varieties that otherwise would not cross.

Howard and Howard (*6*) state that "natural cross-fertilization is common" in the Punjab, where all wheat is grown under irrigation, but is "exceedingly rare in nonirrigated [humid] tracts." The following explanation is offered:

> Under canal irrigation in the Punjab wheat is usually watered twice after sowing, the last watering taking place after the plants are in ear. Often before the last irrigation the supply of water in the soil is so small that the plants partly wilt during the hottest part of the day, the glumes open and the stigmas are exposed to the air. Under such circumstances in the dry hot climates of the Punjab, cross-fertilization is easy, and it is therefore not surprising that it is so frequent.

These conditions certainly would seldom operate at Arlington Experiment Farm in causing natural crossing in wheat. Even in the driest seasons it has not been observed that wilting of wheat plants has occurred at the blooming period. Usually at this time the soil contains sufficient moisture for use of the wheat plant and often there is an oversupply.

The experiments in natural crossing conducted from 1924 to 1926 (Table 5) show that Nebraska No. 28 was cross-pollinated by all varieties or species of Triticum except Black Winter emmer, which flowered too late. Nebraska No. 28 was the first variety to bloom (Table 4), and the last blooming was observed in it the day Alstroum spelt was first observed in bloom; yet eight natural F_1 hybrids between Nebraska No. 28 and Alstroum were present in 1925 in the plants from Nebraska No. 28 seed.

The duration of the flowering period of a variety is influenced by weather and by the number of tillers produced by the plants. In bright, hot weather a variety sown at the rate of 6 pecks per acre on fertile soil will complete its blooming in four days; but in 1924 the blooming periods were prolonged, Nebraska No. 28 requiring 11 days. This was due to the frequent rains and cooler weather and made crossing possible between sorts in which it is normally excluded, such as Nebraska No. 28 and the spelts. Unpollinated stigmas may remain receptive to pollen, however, for several days after the time of dehiscence of the anthers, and early and late varieties may occasionally cross-pollinate under such conditions.

The receptivity of the stigmas of emasculated flowers on heads of winter wheat, representing three-year average data, is shown in Table 9. The data show the percentage of seed set in artificial crosses of common wheat varieties in heads from which the two basal and the tip spikelets and all except the two lower flowers of other spikelets had been removed. The technic reduced the duration of the receptivity of the head, but more than 40 per cent of the flowers of a spike were receptive six days after the first flowering of the spike, and 9 per cent were still receptive on the eighth day. However, the pollen of a head with a similar number of flowers would be shed in two or three days. This difference between the availability of pollen and the receptivity of the stigma is probably

a factor in the nonoccurrence of reciprocal natural hybrids observed in some cases.

The two spelts Alstroum and White Bearded were the pollen parents in 58 natural hybrids with wheat, but on the other hand only White Bearded was the pistillate parent in natural hybrids with wheat, and of these only two occurred. Inasmuch as there was no apparent natural crossing between the two spelt varieties, which bloomed at approximately the same date and were grown adjacent to each other, it may be that these spelts are highly self-fertile.

TABLE 9.—*Receptivity of the stigmas of common wheats, at different ages before and after the first dehiscence of the anthers on a head, to pollen of other wheat varieties during the years 1921, 1922, and 1923 at Arlington Experiment Farm*

Period before or after the first dehiscence of anthers	Number of flowers emasculated and pollinated	Percentage of seed set [a]	Period before or after the first dehiscence of anthers	Number of flowers emasculated and pollinated	Percentage of seed set [a]
1 day	71	3	6 days	199	43
0 day	174	23	7 days	205	19
1 day	134	58	8 days	181	9
2 days	204	53	9 days	153	0
3 days	211	61	10 days	99	0
4 days	192	63	11 days	121	0
5 days	146	42	12 days	80	0

[a] The seed obtained was grown to detect any possible selfing.

Varietal differences to cross-pollination may be the expression of the environment. Kanred and Nebraska No. 28 hybridize naturally and on a significant scale with other varieties at Arlington. These two varieties are poorly adapted to eastern conditions, even to the extent of reduced pollen production; but grown in regions where they are adapted, a different reaction to natural crossing could be expected. The primary culms of Kanred and Nebraska No. 28 are hardly comparable in vigor and flower development to the secondary tillers of such adapted varieties as Purplestraw and Red Rock. The secondary tillers of five common wheat varieties produced on an average almost six times as many natural hybrid plants as the primary culms. All five varieties showed the same phenomenon. To a small extent the greater constancy of the variety from seed of the primary heads may be due to the abundance of pollen of its own kind which could cross-pollinate any flowers escaping self-pollination. The secondary heads, being later and more irregular in flowering, would not have the same opportunity for cross-pollination with wind-borne pollen of their own kind. However, it is highly probable that self-sterility of the flowers of the secondary spikes is greater than that of the primary ones. Flowers are often observed in which the anthers are poorly developed and either do not contain pollen or fail to dehisce, but it is known that in many cases the egg cells of these flowers are capable of being cross-fertilized. This phenomenon of greater pollen sterility in comparison with egg sterility is commonly experienced in the F_1 and later generations of both the wheat-rye and the Aegilops-wheat hybrids.

Kanred and Nebraska No. 28 have both been discarded from the varietal plot experiments at Arlington Experiment Farm, as neither possesses yield merit, nor is it possible in all years to maintain purity to varietal type without roguing to an extent that affects the yield of the plot. Dawson, a white-kerneled wheat, can not be kept pure for seed character without continued selection. Similar difficulty is experienced in maintaining varietal purity in Genesee Giant, Brown Fife, Shepherd, and others, owing to the dominance of certain characters possessed by these varieties. The impurity of the varieties is in part due to mechanical mixtures, but in addition the data accumulated show that natural crossing may be of great importance.

A practical consideration in the conduct of varietal tests is emphasized by these studies of natural crossing in wheat varieties. It has been the practice at Arlington Experiment Farm to grow varieties dissimilar in spike characters adjacent to one another. This permits the separation of bundles accidentally misplaced in harvesting or by windstorms while in the shock. As the plots are usually harvested and threshed in the same order as they are sown, any accidental mixtures in these operations can more easily be detected. In like manner natural crossings are more likely to be evident. When the characters possessed by a variety through their dominance obscure the F_1 natural hybrids that are present, the evidence of natural crossing would not be apparent until the following year when segregation occurred. Roguing would fail to remove the hybrid forms and the segregates approaching the dominant type. Grouping of similar varieties, or of strains of a single type, such as those of Fulcaster or Mediterranean or Poole, together in the field would practically obscure both mechanical mixtures and mixtures resulting from natural crossing. For example, in a field grouping of Poole, China, Shepherd, and Illini Chief, all awnless with glabrous red chaff and purple straw, roguing would remove neither mechanical mixtures nor natural hybrids between any of these varieties. If the natural crossing or mixture was at all extensive, a variety might lose its identity and react in yield and other tests in a manner meaningless so far as varietal significance is concerned.

The necessity of precautions to insure purity of parental types and the selfing of hybrid material for the conduct of genetic investigations has been recognized at Arlington Experiment Farm. This was pointedly brought out in a genetic study of a character transposed by hybridization from rye to a wheat segregate. The segregate exhibits some degree of self-sterility, and the appearance of as high as 15 per cent of hybrid forms in the strain is expected when selfing the previous season has not been practiced. Hayes, Parker, and Kurtzweil (*5*) give data indicating that 18.5 per cent of natural cross hybridization had occurred in F_2 segregates from crosses of *Triticum dicoccum* × *T. vulgare* and *T. vulgare* × *T. durum*.

Carman (*1*) states of the F_3 generation of open-pollinated wheat-rye hybrids:

> It is a matter of very great surprise to us that in this plot there is such a variety of heads that if evidence were suddenly placed before us that all of the varieties of wheat in cultivation sprang from accidental crosses between rye and wheat, we should accept it as in harmony with the appearance of these plants.

No doubt the variety of head types was due to the natural back crossing of the F_1 and F_2 generations with different wheat varieties. In the three cases cited there is present a degree of self-sterility favoring natural crossing which would not be expected in crosses within a species. However, varieties may show self-sterility due to poor vegetative development or pollen abortion, especially in the later tillers, thus giving under certain conditions a high percentage of natural crossing.

The segregation data given in Table 5 probably have been affected to some extent by cross-pollination of the F_1 plants. Several undoubted examples were present in the F_2 material, as, for instance, an awnless red pubescent-glumed plant in a segregating population of Nebraska No. 28 × White Bearded spelt. The awn ratio in the segregating generation of the natural hybrids between the awnless and bearded wheat varieties does not always show a very close fit to the expected. In the segregation of spelt-wheat crosses the awn segregation, however, is 3 to 1. The spelt-wheat natural hybrids consistently produced in the segregating generation a varied assortment of head shapes varying from the linear-fusiform spelt type to a clavate wheat type. The clavate wheat type of head appeared in the crosses of all six wheat varieties with the spelts, though none of the wheat varieties had any inclination toward clavateness, and the appearance of this type of head probably is due to recombination rather than natural crossing of the F_1. No spelt forms exhibiting any degree of clavateness were present.

Whether or not it is necessary to protect the plants from natural crossing in genetic investigations probably will depend on the character or characters studied and also on the locality where the material is grown. If, however, unusual ratios, transgressive segregation, or unexpected types are obtained, the probability of contamination by foreign pollen should be considered.

It is very evident from the data presented herewith that natural crossing may be prevalent in wheat. The high percentages recorded in some cases are surprising even to the writers who have recorded the data, and will be even more so to those who are accustomed to think of wheat as a self-fertilized plant. But in fact it is quite probable that only a part of the crossing actually occurring has been detected. Crossing between different plants of the same variety and between plants of varieties with similar head characters would not be evident either in the F_1 or in later generations. It is rather disconcerting to the experimenter to find that this condition obtains or may obtain. To find that it is necessary to protect from crossing material believed to be essentially self-fertilized must add to the difficulties of experimentation or result in uncertainty when such protection is not practiced.

In the light of these results the conception of a field of wheat as being made up of strains self-fertilized through innumerable generations may need modification. With crossing here and there between plants of like and unlike strains, and with occasional plants of other varieties occurring as mixtures, a field of wheat is likely to be a heterozygous aggregate rather than a homogeneous entity.

SUMMARY AND CONCLUSIONS

Natural hybridization in wheat has been studied at Arlington Experiment Farm during the 10-year period 1917 to 1926, inclusive. From the segregation of plants selected in the years 1917, 1920, and 1924 it is known that natural crossing was frequent in these years.

In some varieties only small amounts of crossing were evident; in others varying percentages were found with a maximum of about 34 per cent occurring in a strain of Fulcaster in 1917.

Varietal differences as regards natural crossing are believed to be due often to environmental conditions. Nebraska No. 28 and Kanred, which are annually cross-fertilized by other varieties, are both poorly adapted to conditions at the Arlington farm.

Extensive natural crossing occurred both in dry and wet blooming periods.

In 1924 approximately six times as much natural crossing occurred in the secondary heads of five common wheat varieties as in the primary heads.

Two spelts grown in a specially arranged sowing in 1924 were almost entirely self-fertilized but were the pollen parents in 58 natural hybrids with common wheat.

Where natural crossing occurs, the field order of the varieties grown in plots should be arranged to favor the expression rather than the obscuring of the crossing.

Precautions to insure selfing may be necessary in the conduct of certain genetic investigations in wheat.

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THE RELATION OF ATMOSPHERIC HUMIDITY TO THE DETERIORATION OF EVAPORATED APPLES IN STORAGE[1]

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INTRODUCTION

One of the most serious problems encountered in connection with the drying of fruits and vegetables is that of deterioration in storage. Various fruits and vegetables behave differently in this respect as a result of a variety of causes. The wide differences in chemical composition of the materials, as in water content, nature and amount of carbohydrate and protein, acidity, tannin content, presence or absence of fats and essential oils, and enzyme content, are responsible for some of the diversity. The differences in treatment given the material in preparation for drying and in the conditions during the drying of the various products are also factors productive of differences in the subsequent behavior of dried materials in storage. Since there are no defined standards for moisture content in the various products, except in the case of evaporated apples, various lots of any given product usually vary considerably in moisture content, not only when stored in bulk prior to packing but also in the final package. In consequence such materials show differences in rate and character of the changes occurring in storage when held under uniform conditions. On the other hand, a product prepared under carefully controlled conditions develops wide differences in appearance, market grade, and palatability, as a result of storage under varied conditions.

With any given dried product the storage factors which are of particular importance are humidity, temperature, and the readiness with which oxygen can gain access to the material. In the case of dried fruits the humidity of the storage chamber and the initial moisture content of the material are factors of outstanding importance in the control of deterioration in storage.

REVIEW OF LITERATURE

Very little work on the deterioration of evaporated fruits and vegetables under controlled conditions is to be found in the literature.

McGillivray (*8*)[2] reported results of studies on moisture content of evaporated apples extending over a number of years. Commercially packed fruit with a content of moisture in excess of 27 per cent at time of boxing in practically every case showed fermentation within six weeks after packing. Experimental packs of fruit having 27 per cent moisture content were placed in cold storage for 16

[1] Received for publication Aug. 15, 1927; issued December, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 905.

months. At the end of that time the moisture content had risen to 34.3 per cent and the material was in considerable part decayed. Similar boxes stored in an office room meanwhile lost considerable weight. When opened, restored to the original weight by sprinkling with sterile water, and repacked, such packages invariably underwent fermentation. This result led McGillivray to condemn very strongly the practice of adding water to dry stock in packing as invariably productive of spoilage. His work led to the adoption of 25 per cent as the permissible moisture content of evaporated apples in Canada.

Prescott, Nichols, and Powers (*12*) conducted investigations of the spoilage of dehydrated vegetables under various conditions of storage. They isolated a large number of species of bacteria and mold fungi, mainly of the commoner soil forms. Storage in very dry air, at either high or low temperatures, resulted in great reduction in the number of bacteria present. At humidities above 70 per cent and temperatures of 20° to 25° C. various vegetables which had been dried to a residual moisture content of 4 per cent absorbed sufficient moisture from the atmosphere during 7 to 10 weeks of storage to permit rapid development of mold colonies. These investigators concluded that evaporated vegetables could be safely stored only in air-tight containers.

Gore and Mangels (*5*) found that when turnips, onions, spinach, and cabbage were dried without previous cooking and stored in sealed glass jars in the dark at 70° to 90° F., the moisture content of the material had to be reduced to 5 per cent in order to preserve odor and color unchanged for six months. With moisture content above this limit, darkening, loss of distinctive odor, and development of distinctly unpleasant odors occurred.

Nichols (*9*) states that "active spoilage" (evidently referring to visible growth of molds) in leaf vegetables such as spinach occurs when the moisture content has reached about 20 per cent, and that starchy and sugary vegetables and fruits generally do not show such spoilage until a moisture content of 25 to 30 per cent has been reached. Enzymic darkening occurred when the moisture content was above 10 per cent but was arrested when moisture was reduced to 5 per cent. Nichols, Powers, Gross, and Noel (*10*) conclude that "to assure best keeping qualities the moisture content of fruits containing much sugar should not exceed 15 to 20 per cent, while that of other fruits and vegetables should not exceed 5 to 10 per cent, the preference in both cases being for the lower percentage." They emphasize the necessity for employing sealable moisture-proof packages for such products.

Cruess, Christie, and Flossfeder (*2*) and Cruess and Christie (*1*) state that grapes dried to 23 per cent moisture after sulphuring will keep indefinitely, but that those with higher moisture contents ultimately mold, as do prunes with more than 25 per cent moisture. Lewis, Brown, and Barss (*7*) state that in their experimental work with prunes they have adopted 17 to 18 per cent as the proper moisture content, but that in some cases it ran as high as 22 per cent without apparent injury to keeping quality.

It is obvious that in some cases the differences in quantities of residual moisture considered safe by different workers are attributable

to differences in climatic conditions under which the experimental work was done, as in the recommendations for prunes just cited. Despite such minor differences, all workers agree that safety in prolonged storage requires drying of all products to somewhat lower moisture contents than are generally employed in commercial practice.

EXPERIMENTAL WORK

The present work was undertaken for the purpose of studying the effect of humidity of the storage room as a factor in the deterioration of dried fruits. Of the various important dried fruits, evaporated apples probably undergo most rapid decline in appearance and quality when stored for considerable periods, despite the fact that a definite limit of moisture content of 24 per cent is prescribed by Food Inspection Decision 176 (*6*). Consequently it was determined to study in some detail the relation of humidity to the changes occurring in this product in storage.

MATERIAL AND METHODS

The fruit employed was grown in the variety orchard of the Office of Horticulture at the Arlington Experiment Farm, Rosslyn, Va. Five varieties—Ben Davis, Delicious, Rome Beauty, Winesap, and Yellow Transparent—were used. These were chosen by reason of their differences in chemical composition and in color and texture of flesh. Four of the five are employed in some quantity in the commercial production of evaporated apples. Yellow Transparent, which is not used in commercial drying, was also used for the reason that it differs considerably in composition from the others. It is a white-fleshed, soft-textured summer variety, high in acid and astringent content, low in sugar and total solids, and has little distinctive flavor. Ben Davis is a coarse-fleshed white variety, rather low in sugar content for a variety of its season, medium in acidity and astringency, and has little distinctive flavor. Delicious is a yellow-fleshed variety of medium sugar content, low in astringency and exceptionally low in acid content, and has a highly distinctive flavor. Rome Beauty has flesh slightly tinged with yellow, a medium sugar content, low acidity, and a fairly pronounced and characteristic flavor. Winesap has a somewhat yellow, fine-grained flesh, high in sugar content, is of medium acidity and astringency, and has a distinctive flavor.

No analyses of the whole fruits were made, but portions of the same lots were pressed for juice making, and analyses of the freshly expressed juices were made. The results of these analyses, which convey an idea of the particular lots of fruit employed, are recorded in Table 1.[3]

[3] The volumetric permanganate method was employed in determining free reducing sugars and total sugars after acid hydrolysis. Acidity was determined by titration with N/10 sodium hydroxide against phenolphthalein, the results being calculated as malic acid. Total and nontannin astringency were determined by the Loewenthal-Procter method. Total solids were determined by drying portions of the fresh juice to moist dryness on a water bath and bringing to constant weight in a vacuum oven at 80° C. Hydrogen-ion concentrations were made by the use of the Bailey hydrogen electrode, and were checked with the quinhydrone electrode.

TABLE 1.—*Chemical composition of freshly expressed juices of apple material used in storage experiments*

Variety	Constituents (per cent)								
	Acid as malic	Reducing sugar	Cane sugar	Total sugar	Total astringency	Tannins	Non-tannins	Total solids	Hydrogen-ion concentration
Ben Davis	0.520	6.32	3.42	9.74	0.924	0.436	0.488	13.44	3.40
Delicious	.279	9.00	1.10	10.10	.868	.377	.491	12.46	4.02
Rome Beauty	.360	6.62	3.79	10.41	.817	.204	.613	12.89	3.54
Winesap	.520	8.40	4.61	13.01	.859	.430	.429	15.07	3.43
Yellow Transparent	.680	6.84	1.06	7.90	1.942	1.125	.817	9.23	3.36

The fruit used was a portion of the tree-run crop of the varieties harvested when commercially picking ripe and was delivered to the laboratory as soon as picked. It was stored in a basement room until it had reached firm eating-ripe condition. A considerable quantity of each variety was then peeled, cored, and sliced by machine, and the sliced fruit was divided into four portions. One lot was immediately placed in the evaporator without further treatment. A second lot was spread on drying trays and heated to 80° C. (176° F.) for 15 minutes by placing in a closed chamber heated by steam jets placed beneath the trays, then transferred to the evaporator. A third lot was dipped into 2 per cent cold sodium chloride solution for 10 to 15 seconds before spreading on the drying trays, and a fourth lot was exposed on trays to the fumes of burning sulphur in a closed chamber for 30 minutes. All were dried together in a tunnel evaporator at a temperature of approximately 60° to 65° C. (140° to 149° F.).

The four treatments described, when applied to fruit of the five varieties employed, yielded material showing a very wide range in appearance and color as it was taken from the evaporator. The material which received no treatment prior to drying showed considerable brown discoloration at the surface of the slices as a result of oxidations during drying. This was much more pronounced in Yellow Transparent than in the other varieties. The untreated lots in all varieties were distinctly superior to the others in flavor, but considerably less attractive in appearance and color. The material treated with sulphur dioxide was the most attractive in appearance, as it showed no browning or other discoloration. The color was a bright light yellow in Ben Davis and Yellow Transparent and a slightly deeper golden yellow in Delicious, Winesap, and Rome Beauty. The flavor of the sulphured material was very good but scarcely equal to that of the untreated material.

The material treated with sodium chloride was much whiter than that receiving any other treatment, but had a peculiar grayish-white tint. Occasional pieces had a very faint pink discoloration, especially noticeable in Yellow Transparent and Winesap. The flavor was distinctly inferior to that of the sulphured material, but the slight saltiness of this fruit made comparison of the two lots somewhat difficult.

The material heated to 80° C. (176° F.) in a current of steam was considerably brighter in color than untreated material, but much

less attractive in appearance than that treated with sulphur fumes or sodium chloride. Many of the slices collapsed during the drying, becoming thin and semitransparent and adhering firmly to the drying trays. The material was distinctly lacking in the richness and fullness of flavor possessed by the untreated material, but was superior in this respect to that which had been dipped in sodium chloride.

All the lots of fruit were allowed to remain in the evaporator until the pieces contained approximately 15 per cent of moisture. The samples used in the storage experiment consisted of 100 gm. each in one series and 200 gm. in another, and were weighed out in a tared dish and immediately transferred to muslin bags and placed in the storage chambers. Three to five identical samples were prepared from each lot of fruit, in order to permit withdrawal of samples for examination and determination of moisture content at intervals during the experiment.

The storage chambers consisted of tall bell jars of 11,000 c. c. capacity, held in inverted position by suitable supports and covered by heavy glass plates made air-tight by stopcock grease. Atmospheric humidities ranging from 88.8 to 8.5 per cent by steps of approximately 10 per cent were obtained by placing in each of the chambers a comparatively large quantity (1,850 c. c.) of sulphuric acid solution of the proper concentration. The series was completed by adding two chambers, one containing calcium oxide, the other distilled water. This gave a series of 10 chambers having relative humidities respectively of 0, 8.5, 18.8, 37.1, 47.7, 58.3, 70.4, 80.5, 88.8, and 100 per cent.

The samples of fruit were loosely packed into small muslin bags, and the bags placed on perforated porcelain plates supported at some distance above the level of the liquid in the chambers. Care was taken to arrange the bags so that free access of air to all the samples was not restricted. The purpose of the experiment was to simulate the conditions prevailing in fruit stored loosely in bulk, and not those in material packed in final containers.

The series of chambers were placed in an insulated room kept at a constant temperature of 25° C. (77° F.) for the first 12 months of storage, after which it was necessary to transfer them to a laboratory room in which the seasonal fluctuations of temperature ranged between 20° and 30° C. (68° and 86° F.). Since it was possible to employ only one controlled temperature, that chosen was made to approximate the maximum temperatures encountered in common storage. Temperature is a factor of considerable importance in determining the rate of deterioration, so that the rate and extent of deterioration here observed would not be expected to hold in experiments carried on at other temperatures.

The material was examined at intervals of one to two weeks during the first two or three months of storage and at longer intervals thereafter. Detailed notes were made at each examination as to any discoverable changes in appearance, color, and market grade of the material, and at intervals of three or four months one of the duplicate samples of each lot was removed and employed for a determination of moisture content after any changes in color, odor, flavor, and palatability had been determined.

CHANGES IN APPEARANCE AND PALATABILITY

Alteration in appearance was rather promptly apparent in the fruit in the chambers having high humidities. In the chamber with saturated atmosphere a slight brownish discoloration could be observed in the material after 10 days, and this continued to deepen until all the material was uniformly dark brown in color. This change occurred somewhat less rapidly in the material treated with sulphur dioxide than in that receiving other treatments, but at the end of six to eight weeks the sulphured material was indistinguishable from the other lots. Of the five varieties employed, Yellow Transparent showed discoloration earlier than the others and also developed it somewhat more rapidly.

In the chamber with 88.8 per cent humidity the changes were in all respects identical, with the single difference that they appeared a little later and developed somewhat more slowly. As the material from the series of chambers was examined the alteration in color became less and less evident until a point was reached at which no change in color was apparent. After six weeks' storage this point of no change was reached in the chamber having 70.4 per cent relative humidity, but with continuation of the storage period it shifted to a lower humidity, as indicated in Tables 2 and 3.

Concurrently with the changes in color, alterations in the moisture content, odor, and flavor of the material began to appear. Even at the end of 10 days it was apparent that the material in 100, 88.8, and 80.5 per cent humidity was gaining in weight through absorption of moisture, and this became increasingly apparent in these lots at subsequent examinations. At the end of six weeks the development of mold colonies was evident on occasional slices in all but the sulphured lots in the saturated atmosphere, and the odor of incipient fermentation was apparent on opening both the 100 per cent and the 88.8 per cent chambers. A slightly bitter flavor was apparent in the material dried without any treatment and kept in 100 and 88.8 per cent humidity. These changes became less evident in passing down the scale of humidity, the material in the 70.4 per cent chamber having undergone no discoverable change in color, odor, or flavor, and little if any change in moisture content at the end of six weeks.

In the case of the lots stored at humidities less than 70.4 per cent, it was apparent after six weeks that loss of moisture had occurred in quantities proportional to the relative humidities of the various chambers, but the odor, flavor, and color of the material was unchanged.

In the interval between the sixth and sixteenth weeks of storage, the changes occurring may be summarized as consisting of the appearance of some degree of discoloration in the chambers having 70.4, 58.3, and 47.2 per cent humidity, in the order named, followed by the development of a musty, unpleasant odor and, in the case of the material at higher humidities, of a noticeable alteration in taste. In the 100 and 88.8 per cent chambers absorption of moisture and continued growth of molds and yeasts converted the material within the bags to soggy, decaying masses having a strong odor of fermentation.

TABLE 2.—*Condition of evaporated Yellow Transparent apples after 112 days' storage at various humidities*

Humidity	Treatment	Presence of molds	Taste	Odor or flavor	Color	General appearance	Grade
Saturation	Untreated	Abundant	Bitter, rancid	Musty, fermenting	Dirty brown	Very poor	Unmarketable.
Do	SO_2	do	do	do	Very brown	do	Do.
Do	NaCl	do	do	do	do	do	Do.
Do	Steam	do	do	do	Dirty brown	do	Do.
88.8 per cent	Untreated	Small quantity	do	Very musty	Very brown	do	Do.
Do	SO_2	do	do	do	do	do	Do.
Do	NaCl	do	do	do	do	do	Do.
Do	Steam	do	do	do	do	do	Do.
80.5 per cent	Untreated	Occasional	Bitter, stale	do	do	do	Do
Do	SO_2	do	do	do	do	do	Do.
Do	NaCl	do	do	do	do	do	Do.
Do	Steam	do	do	do	do	do	Do.
70.4 per cent	Untreated	None	Somewhat stale	Old sugar-barrel odor	do	do	Do.
Do	SO_2	do	do	do	do	do	Do.
Do	NaCl	do	do	do	do	do	Low prime.
Do	Steam	do	do	do	do	do	Unmarketable.
58.3 per cent	Untreated	do	do	do	do	do	Scarcely marketable.
Do	SO_2	do	do	do	do	Medium	Good prime.
Do	NaCl	do	do	do	do	do	Prime.
Do	Steam	do	do	do	do	Poor	Low prime.
47.2 per cent	Untreated	do	A little stale	Somewhat abnormal	do	Medium	Do.
Do	SO_2	do	do	do	Light brown	Good	Good prime.
Do	NaCl	do	do	do	Grayish brown	Medium	Do.
Do	Steam	do	do	do	do	Poor	Low prime.
37.1 per cent	Untreated	do	Fair	Almost normal	do	Medium	Do.
Do	SO_2	do	do	do	Light brown	Good	Good prime.
Do	NaCl	do	do	do	Grayish brown	Medium	Do.
Do	Steam	do	do	Very good	do	do	Low prime.
18.8 per cent	Untreated	do	Fairly good	do	do	Good	Do.
Do	SO_2	do	do	do	Bright light yellow	Bright light color	Good prime.
Do	NaCl	do	do	do	Grayish pink	Medium	Do.
Do	Steam	do	Fair	do	Light brown	Fair	Do.
8.5 per cent	Untreated	do	Good	do	do	Good	Do.
Do	SO_2	do	do	do	Bright light yellow	Very good	Choice.
Do	NaCl	do	Fair	do	Somewhat pink; grayish	Fair	Prime.
Do	Steam	do	do	do	Somewhat brown	Good	Good prime.
Completely dry	Untreated	do	do	do	do	do	Prime.
Do	SO_2	do	do	do	Bright light yellow	do	Good prime.
Do	NaCl	do	do	do	Grayish	Fair	Do.
Do	Steam	do	do	do	Light brown	Good	Do.

TABLE 3.—*Condition of evaporated Rome Beauty apples after 112 days' storage at various humidities*

Humidity	Treatment	Presence of molds	Taste	Odor or flavor	Color	General appearance	Grade
Saturation	Untreated	Present	Stale, acid rancidity.	Very musty	Very dark brown	Very poor	Unmarketable.
Do	SO_2	do	do	do	do	do	Do.
Do	NaCl	do	do	do	do	do	Do.
Do	Steam	do	do	do	do	do	Do.
88.8 per cent	Untreated	Small amount	Very stale	do	Dark brown	do	Do.
Do	SO_2	do	do	do	do	do	Do.
Do	NaCl	do	do	do	do	do	Do.
Do	Steam	do	do	do	do	do	Do.
80.5 per cent	Untreated	Occasional	Stale	Old sugar-barrel odor	Brown	Poor	Scarcely marketable.
Do	SO_2	do	do	do	do	do	Low prime.
Do	NaCl	do	do	do	do	do	Do.
Do	Steam	do	do	do	do	do	Scarcely marketable.
70.4 per cent	Untreated	None	do	do	do	do	Do.
Do	SO_2	do	do	do	do	Medium	Do.
Do	NaCl	do	do	do	ght brown	do	Low prime.
Do	Steam	do	do	do	Brown	Poor	Marketable.
58.3 per cent	Untreated	do	Somewhat stale	Somewhat abnormal	do	Medium	Low prime.
Do	SO_2	do	do	do	Light brown	Fair	Prime.
Do	NaCl	do	do	do	Pinkish brown	Poor	Do.
Do	Steam	do	do	do	Brown	do	Low prime.
47.2 per cent	Untreated	do	Fair	Fair	do	Medium	Do.
Do	SO_2	do	do	do	Slightly brown	Fair	Good prime.
Do	NaCl	do	Poor	do	Grayish pink	Poor	Prime.
Do	Steam	do	do	do	Somewhat brown	do	Low prime.
37.1 per cent	Untreated	do	Excellent	Good	do	Fair	Prime.
Do	SO_2	do	do	do	Light yellow	Excellent	Good choice.
Do	NaCl	do	Fair	do	Grayish pink	Abnormal	Prime.
Do	Steam	do	Good	do	Somewhat brown	Poor	Do.
18.8 per cent	Untreated	do	Excellent	Very good	do	Fair	Do.
Do	SO_2	do	do	do	Bright, light yellow	Excellent	Fancy.
Do	NaCl	do	Fair	do	Pinkish	Abnormal	Choice.
Do	Steam	do	Good	do	Slightly brown	Fair	Do.
8.5 per cent	Untreated	do	Excellent	do	Somewhat brown	Good	High prime.
Do	SO_2	do	do	do	Bright, light yellow	Excellent	Extra fancy.
Do	NaCl	do	Fair	do	Rose pink	Somewhat normal	Choice.
Do	Steam	do	Good	do	Slightly brown	Fair	Do.
Completely dry	Untreated	do	Excellent	do	Somewhat brown	Good	High prime.
Do	SO_2	do	do	do	Bright, light yellow	Excellent	Extra fancy.
Do	NaCl	do	Fair	do	Greenish, slightly pink	Fair	Choice.
Do	Steam	do	Good	do	Slightly brown	do	Do.

The condition of the materials in the various chambers at the end of 112 days of storage is stated in summary form for two of the varieties, Yellow Transparent and Rome Beauty, in Tables 2 and 3. The conditions obtaining in the other varieties were so closely identical with those in Rome Beauty that they do not need detailed separate statements, but a few minor differences will be noted in a subsequent paragraph.

In addition to the statements tabulated in Tables 2 and 3 it may be noted that at the end of 112 days of storage all the material stored at humidities of 47.2 per cent or higher had become rather uniformly brown, regardless of the nature of the treatment given prior to drying. Thus the material treated with sulphur dioxide, while not developing discoloration as early as the other lots, at the end of this time had become indistinguishable from the fruit receiving no treatment prior to drying.

At relative humidities lower than 47.2 per cent there was progressive improvement in color with decrease in the atmospheric moisture of the storage chamber in the case of the sulphured material, and at 18 per cent humidity or less there was no change in color. Untreated material was distinctly browned at all humidities above 18 per cent, while that which had been steamed prior to drying was also browned, but to a lesser degree than untreated material. The material dipped in sodium chloride solution was indistinguishable from that receiving other treatments in the case of the lots stored at higher humidities, but in the material held at lower humidities a distinctly pinkish coloration was present. This was not observed except in material treated with sodium chloride, but it was present in all such material stored in relatively dry air. In the case of Yellow Transparent this pink coloration was first observable in the fruit held at 37.1 per cent humidity and was most pronounced in that held at 18.1 per cent. In the other varieties it was detectable in the salt-treated material held at 58.3 per cent as a pinkish tinge along the core line and about the vascular bundles of the slices. In material held at 47.2 per cent humidity all the slices were grayish pink at the surfaces but showed a central zone of normal color when cut across. The pink discoloration was still more pronounced in material held at 37.1 per cent and was present in decreasing degree in the material held at lower percentages of atmospheric moisture. The fruit of the different varieties differed somewhat in that the pink color was more pronounced in those which had whitest flesh.

It would appear probable that this pink discoloration is the result of reaction of the absorbed sodium chloride with some constituent of the apple flesh, probably a flavone or tannin. Whatever the nature of the reaction responsible for the development of the pink color, it is peculiar in that the color is most strongly developed in an atmosphere having 18 to 37 per cent relative humidity and decreases in intensity with either increase or decrease in humidity of the storage room. This type of discoloration has been observed by one of the writers in apples evaporated after dipping in sodium chloride and subsequently stored in a very dry atmosphere, and also in overdried commercial evaporated apples which had been salt dipped prior to drying, but it does not seem to have been mentioned in the literature.

From the results obtained with the use of sulphur dioxide and sodium chloride it may be concluded that these agents are effective in

preventing brown discoloration during the drying process, but that the effect is temporary in character. Neither of these agents prevents a gradual browning of the tissues, which ultimately appears in storage, although sulphur dioxide delayed its appearance somewhat longer than sodium chloride under the conditions of these experiments. This is in general agreement with the results of Overholser and Cruess (*11*) on the effectiveness of these treatments in preventing discoloration in apple juices. Those authors did not investigate the effect of prolonged storage.

The effects of varying degrees of humidity upon changes in odor and flavor of the fruit were of about the same order as the effects upon alteration of color, as indicated in Tables 2 and 3, but developed more slowly. All the samples held at humidities lower than 80.5 per cent remained normal in odor and taste during the first six weeks of storage. Those at higher humidities developed colonies of molds and were markedly abnormal in odor and taste. At the end of 112 days all samples at humidities higher than 70.5 per cent had developed a distinctly bitter, unpleasant taste and an odor strongly suggestive of barrels in which moist, unrefined sugar had been stored. These abnormalities in odor and flavor were perceptible in samples in which no visible growth of mold or other organisms had occurred, as in some of the lots from the 80.5 and 70.4 per cent chambers. In the lots held at humidities below 70.4 per cent, odor and taste became progressively more nearly normal with decreasing humidity, and at a humidity of 37 per cent or less no detectable change had occurred.

Yellow Transparent differed somewhat markedly from the other varieties in that it showed greater alteration in color, odor, and flavor under any given condition than did the other varieties. In other work it has been observed that most early apples of the low-sugar, high-acid types are much like Yellow Transparent, deteriorating rapidly in storage. This fact is familiar to many operators of commercial evaporators, who base their refusal to employ early maturing varieties upon the impossibility of preserving the appearance and quality of the material for more than very short periods.

The behavior of Ben Davis, Delicious, Winesap, and Rome Beauty with respect to alteration in color, odor, and flavor under the various conditions of storage was so closely identical that details with respect to Rome Beauty (Table 3) are fairly representative of the group. The fall and winter varieties, with their relatively higher sugar content, are much better preserved in storage than is Yellow Transparent. The relatively low sugar content and the high tannin content of the variety last named appear to offer conditions favorable to rapid oxidation and other changes involved in deterioration in spite of its higher total and actual acidity.

That hydrogen-ion concentration may be an important factor in controlling the rate of action of oxidase is indicated by the work of Overholser and Cruess (*11*), who found that in apple juice darkening on exposure to air was progressively lessened by progressively increasing additions of hydrochloric acid. Falk, McGuire, and Blount (*4*) also found that in potatoes and certain other vegetables, both fresh and dehydrated, the activity of oxidase and peroxidase was progressively inhibited by increasing acidity of the medium. In the present work deterioration was most rapid and greatest in the variety having highest total acidity and hydrogen-ion concentration. This

may indicate that these changes are not produced by enzymes; if they are, other factors outweigh the effect of acidity.

Certain consistent minor differences in the behavior of the dried product made from the different winter varieties which were noted in the course of the work are of sufficient interest to warrant brief statement. At any given degree of humidity, the material of Delicious was invariably darker in color, more abnormal in odor and flavor, and showed more extensive growth of molds, where molds were present, than did samples of Winesap and Ben Davis given identical treatments and stored under the same conditions. Rome Beauty like Delicious showed much greater change in color under given conditions than did Winesap and Ben Davis, but differed in that odor and flavor were as well preserved as in the latter varieties. At the lower humidities, fruit of Delicious and Rome Beauty which had been salt dipped prior to drying showed distinctly less pink discoloration than did salt-dipped Ben Davis or Winesap from the same storage chambers. The behavior of Rome Beauty differed in one respect from that of all the others, in that the brown discoloration developed at the higher humidities was invariably reddish brown, as contrasted with the light to dark brown, without admixture of red, seen in the other varieties. Much more extended work would be necessary to determine whether these differences are constant for the varieties.

FURTHER CHANGES ON EXTENSION OF THE STORAGE PERIOD

As indicated in Tables 2 and 3, the material kept in atmospheric humidities of 100 and 88.8 per cent had been so far altered after 112 days' storage as to be unpalatable and unmarketable. These lots were consequently discarded after samples had been taken for determination of moisture content. The experiment was continued with the remaining chambers, which were held in a room having a constant temperature of 25° C. (77° F.) for one year, then transferred to a room in which the temperatures ranged from 20° to 30° C. (68° to 86° F.) with changes in the season, and there held for a further period of two years and three months. The chambers were opened from time to time for inspection and the making of notes as to condition of the material.

There were no further rapid changes in appearance and color of the material. The lots in atmospheres having humidities of 80.5 to 47.2 per cent underwent a slow, progressive darkening in color, which was most rapid in rate and greatest in degree in the chamber having greatest moisture content and decreased in rate and degree with decrease in humidity of the air present. In the higher humidities there was progressive alteration of odor and flavor, the bitterness and objectionable odor observed earlier in the material stored in approximately saturated atmospheres becoming more and more apparent with the passage of time. These changes were not conditioned upon the development of molds, since they occurred in the absence of any visible growth of organisms. In the chambers having humidities of 37.1 per cent or less the material remained in perfect condition in respect of color, appearance, odor, and flavor, except in the case of Yellow Transparent. In that variety perceptible browning had occurred even in the chamber having 8.5 per cent humidity,

and only the samples held over calcium oxide retained their original color after more than three years.

The experiment was discontinued at the end of three years and three months, when detailed final notes were taken and determinations of moisture content made upon all the lots of material. Minor differences in the several varieties were noted, but these were of the character already described.

MOISTURE CONTENT ATTAINED BY THE MATERIAL AT VARIOUS HUMIDITIES

The moisture-absorbing or water-holding capacity of the material at various degrees of humidity is of particular interest. The moisture content of the various lots of material varied slightly when they were placed in the humidity chambers, but closely approximated 15 per cent. As the material gained or lost in weight the sulphuric-acid solutions became more concentrated or more dilute, with a resulting alteration of the humidity of the chamber from its original calculated content. An error was thus introduced, but the quantity of acid used in each chamber was purposely made large enough to reduce the error to a small value, which was further reduced by replacing the acid after the first 8 or 10 weeks of storage.

Moisture determinations were made by weighing out 50 to 100 gm. of the sample and drying to constant weight at 80° C. in a vacuum oven. Table 4 shows the result of the moisture determinations made at the end of the storage period of three years and three months for the samples held in the lower humidities (70.4 per cent or less) and at the end of shorter periods for those in the higher humidities.

The data for the samples in the high humidity chambers (Table 4) are incomplete for two reasons. Many of the samples showed such extensive attack by molds and yeasts that large losses of sugar had occurred. Such samples were weighed to the nearest gram as they were removed from the chambers, no attempt at more accurate determinations being made. Those showing less extensive attack by organisms were sampled for weighing, but some of the samples were destroyed by accident before final weighings were obtained. The crude weighings show sufficiently close agreement with the figures given in the table to make it certain that these figures are truly representative of the condition of the material in these chambers as regards its water content.

TABLE 4.—*Moisture content of evaporated apples of different varieties held in atmospheres of various humidities after attainment of equilibrium* [a]

Humidity	Treatment	Moisture content (per cent)				
		Yellow Transparent	Rome Beauty	Winesap	Delicious	Ben Davis
Saturation	Untreated	54.31	55.46			
Do	Steam					51.74
Do	NaCl		56.31			
Do	SO_2			55.23		
88.8 per cent	Untreated	33.35	35.18		36.57	42.04
Do	Steam					40.38
Do	NaCl			43.23	44.66	42.00
Do	SO_2				46.99	42.25
80.5 per cent	Untreated	26.56	25.78	25.35		28.63
Do	Steam			24.54		27.70
Do	NaCl		27.03	26.76		29.38
Do	SO_2		24.93	23.20	25.59	27.36
70.4 per cent	Untreated	23.42	20.19	19.27	17.65	20.42
Do	Steam		19.06	19.00		18.66
Do	NaCl		21.28	21.12	22.21	21.18
Do	SO_2		20.59	20.22	21.59	19.73
58.3 per cent	Untreated	13.69	14.38	13.28	13.74	13.25
Do	Steam	13.90	13.31	13.00		13.58
Do	NaCl	14.85	14.81	14.12	13.74	14.75
Do	SO_2	13.57	14.24	15.36	12.82	14.27
47.2 per cent	Untreated	9.79	9.66	9.50	6.62	9.90
Do	Steam	8.64	8.92	9.00		8.76
Do	NaCl	10.97	10.21	10.26	11.33	10.70
Do	SO_2	8.84		8.47	10.80	10.02
37.1 per cent	Untreated	7.59	7.67	7.76	6.97	6.20
Do	Steam	7.00	6.34	6.57		6.51
Do	NaCl	8.22	7.77	7.45	5.83	7.24
Do	SO_2	7.62	7.74	7.00	7.14	6.71
18.8 per cent	Untreated	4.30	3.87	3.61	2.36	2.95
Do	Steam	3.78	2.69	3.10		2.02
Do	NaCl	4.66	4.03	3.85	2.94	2.26
Do	SO_2	3.80	4.03	3.56	2.39	2.61
8.5 per cent	Untreated	2.42	.15	1.97	.61	.89
Do	Steam	1.57	.11	1.60		.85
Do	NaCl	2.44	.11	1.80	.33	
Do	SO_2	1.78	.57	2.01	.32	.63
Completely dry	Untreated	.40	.53	.47	.74	1.04
Do	Steam	.41	.16	.63		1.04
Do	NaCl	.35	.65	.68	.66	.96
Do	SO_2	.46	.04	.91	.83	.72

[a] In the case of the lower humidities (70.4 per cent or less) the weighings were made at the end of three years and three months. In the higher humidities the weights recorded are the last it was possible to obtain before invasion by molds proceeded so far as to invalidate the weighings.

Evaporated apples are very highly hygroscopic, and this property is the initial cause for deterioration. When stored in air of any given degree of humidity, the material, regardless of its original moisture content, comes into equilibrium with the atmosphere by gain or loss of moisture. In the present instance material having an initial moisture content of 15 per cent was practically in equilibrium with an atmosphere of 58.3 per cent relative humidity, as almost no gain or loss in moisture content occurred. With increase in humidity there was a rather uniform increase in moisture content of the material, that in a saturated atmosphere taking up somewhat more than its own weight of water. With humidities below 58.3 per cent there was progressive decrease in moisture content of the material, that stored over calcium oxide becoming nearly water free. As the presence of a certain quantity of water in the material is one of the necessary conditions for the occurrence of deterioration, the samples having less than this quantity show no discoverable changes even on greatly prolonged storage, and those having more than this quantity

show a rate and degree of alteration very definitely related to their water content.

The highly hygroscopic character of desiccated plant material has not always been sufficiently considered, or certainly has not always been adequately emphasized, in making recommendations as to "safe" moisture contents for evaporated products. There is no "safe" moisture content for any such material unless it is at once placed under such conditions that absorption of water vapor from the atmosphere can not occur. Unless this is done, drying to any arbitrarily chosen percentage of moisture, no matter how low, can be expected to preserve the material unchanged only so long as the moisture of the surrounding atmosphere does not rise above the point of equilibrium with the product. If this occurs, water will be absorbed to an extent determined by the humidity and the freedom of access of air to the material, and spoilage may occur. This fact is sufficiently important to warrant the placing of decided emphasis upon it in any consideration of preservation of materials of hygroscopic character by drying.

Some conception of the magnitude of the forces concerned in the absorption and retention of moisture by partially desiccated fruits may be gained from consideration of the fact that evaporated apples with 13 to 15 per cent moisture are in equilibrium with an atmosphere of about 58.3 per cent relative humidity, which is obtained over sulphuric acid of 1.30 specific gravity (40 per cent acid). Over acid of 1.25 specific gravity (33.5 per cent acid) such material absorbs an additional 5.7 per cent of moisture, while over acid of 1.35 specific gravity (45 per cent acid) it gives up an approximately equal amount. The assumption that fruit of 15 per cent moisture content is approximately in equilibrium as respects its moisture-absorbing power with sulphuric acid of 1.30 specific gravity thus appears to be justified. While the osmotic pressures of sulphuric acid in high concentrations can not be accurately determined, as Shull (*14*) has shown, from the use of formulae for "ideal" solutions, calculations by several methods give values ranging from 720 to 798 atmospheres as the osmotic pressure of sulphuric acid of 1.30 specific gravity.

Comparisons of the data on moisture content of material given various treatments prior to drying indicate that such treatments may to some extent affect the water-absorbing capacity of the fruit. That which was dried without treatment and that which had been exposed to sulphur dioxide showed no consistent differences in this respect. That which had been heated to 80° C. in steam rather consistently showed reduced moisture absorption or retention as compared with untreated material, while that dipped in sodium chloride showed somewhat higher moisture absorption, which may be attributed to the salt remaining in and upon the slices.

That the moisture-absorbing capacity of a variety is not directly related to its content is evident from inspection of the data in Tables 1 and 4. Winesap, which is considerably higher in sugar than any of the other varieties, took up smaller percentages of water, especially at the higher humidities, than did the others. Yellow Transparent took up quite as much moisture as any of the others and in a number of cases more, despite its very low content of sugar and total solids. It is evident that the water-absorbing capacity of the several varieties is determined by differences in the character and amount of the

hydrophile colloids present, and that sugar content plays only a minor rôle therein.

A somewhat unexpected outcome of the present work is the discovery that total arrest of deterioration in evaporated apples over prolonged periods can be accomplished only by reducing the moisture content to 8 per cent, as was done in the chamber having 37.1 per cent relative humidity, or a lower figure.

At constant humidities ranging from 47.2 to 80.5 per cent deterioration in color became apparent within a short time and was followed on prolonged storage by development of abnormal, rancid odor and pronounced bitter flavor. At the end of 112 days these changes were well advanced in all the fruit having a moisture content in excess of 18 per cent, and after three years all the material having more than 10 per cent of moisture had become unmarketable if not wholly unfit for consumption. These changes were not accompanied by visible growth of molds or yeasts and occurred under conditions, at least in the lower humidities, which inhibit the growth of these organisms. These changes have been considered as due to the action of enzymes and as being of less importance than those due to molds (*9*). In the apples here used it is questionable whether enzymes are concerned, since they occur in fruit which has been heated to a temperature of 80° C. (176° F.), or in that treated with sulphur dioxide or sodium chloride, to the same degree as in untreated material.

The work of Overholser and Cruess (*11*) indicates that although sulphuring or heating to 80° C. does not inactivate the peroxidase of apple tissue, both treatments destroy the organic peroxide and thus inactivate the oxidizing system. That dilute sodium chloride and other alkali chlorides markedly depress the activity of oxidizing systems is indicated by the work of Ewart (*3*) on the oxidase of apple tissue and by that of Rose, Kraybill, and Rose (*13*) on that of apple bark. If the discoloration of the tissues is the result of enzyme action, the effects of such agents as heat, sulphur dioxide, and sodium chloride upon the oxidizing system are only temporary in character and are followed by ultimate resumption of activity, notwithstanding the continued presence of sulphur dioxide or salt. Furthermore, the action was most rapid in the case of the variety having highest titratable and actual acidity.

Whatever the cause of the darkening in color and the attendant development of abnormal odors and flavors, these changes are less important than those produced by molds and yeasts only in that they occur more slowly, hence require a longer time to reduce the material to unmarketable condition. But they are extremely important, in that they lower the grade and value of the product in which they occur, and are not arrested by bringing the moisture content of the material to a level at which growth of organisms can not occur.

At moisture contents higher than 25 per cent, the deterioration process develops another phase in consequence of the growth and multiplication of organisms which quickly render the material unmarketable and ultimately wholly destroy it.

Adequate protection against deterioration in evaporated apples can be obtained only by taking into consideration the facts in regard to

the two interrelated but independent types of deterioration here described. Temperature is an important factor in determining the rate and extent of deterioration of both types, but the effects of variation in this factor have not been included in this study.

SUMMARY

Evaporated apples of uniform initial moisture content, made from five varieties of rather widely varying types and given four different treatments preparatory to drying, were stored at a temperature of 20° to 30° C. (68° to 86° F.) in atmospheres having humidities ranging from complete saturation to complete dryness for somewhat more than three years, with frequent examination.

The material in all cases either absorbed or gave up moisture until a new moisture content in equilibrium with the moisture of the chamber had been reached.

At relative humidities of 18.8 per cent or less, the material retained its original color, odor, and flavor unchanged throughout the entire storage period. Evaporated apples can be preserved indefinitely by sufficiently reducing the moisture content of both the material and the storage chamber.

At relative humidities between 47 and 80.5 per cent, growth of molds and yeasts did not occur, but the fruit developed progressive brownish discoloration, rancid odor, and abnormal, bitter flavor, which rendered the material unpalatable and unmarketable before the expiration of the storage period. The rate at which these changes occurred increased with increase in humidity throughout the series.

These alterations in color, odor, and flavor became evident within one year in fruit having the moisture content reduced to from 9 to 10 per cent when the material was loosely piled so that air had free access to it. If the changes are produced by enzymes, the enzymes concerned are not destroyed by treatment of the fruit with sulphur dioxide or sodium chloride or by heating it to 80° C. (176° F.) prior to drying.

At relative humidities higher than 80.5 per cent, the absorption of moisture was sufficient to permit rapid and abundant development of molds and yeasts, which destroyed the material within a few months.

Exposure to fumes of sulphur dioxide, immersion in 2 per cent sodium chloride, or heating in steam to 80° C. (176° F.) preparatory to drying, did not prevent the development of discoloration at low humidities or the growth of organisms at the higher humidities, all the lots kept at any given humidity ultimately reaching the condition of the untreated control. Of the various treatments, that with sulphur dioxide retarded the development of these changes somewhat longer than the others. Sodium chloride was next in effectiveness, but its reaction with some constituent of the fruit produced a characteristic alteration of color in some varieties.

The several varieties showed considerable differences in the rate at which deterioration developed, deterioration being most rapid in the variety which combined highest acidity and astringency with lowest content of sugar.

Evaporated apples are highly hygroscopic. Material having 13 to 15 per cent moisture is in approximate equilibrium with the atmosphere over sulphuric acid of 1.30 specific gravity, having an osmotic pressure approximating 750 atmospheres. Fruit having the standard moisture content of 24 per cent is in approximate equilibrium with air of 75 per cent relative humidity at 30° C. (86° F.) and will rapidly absorb moisture when the atmospheric moisture rises above this value.

Adequate protection of evaporated apples against deterioration in prolonged storage involves thorough drying and careful packing of the material and control of the humidity of the storage room. By thorough drying is meant the reduction of contained moisture, not merely to a point at which mold growth can not occur, but to a lower point at which oxidations and other changes not dependent upon the growth of organisms are arrested. Control of humidity in the storage room implies maintenance of atmospheric humidity at or below the point of equilibrium with the material. Careful packing involves the use of the most efficient means of preventing access of atmospheric oxygen to the material.

The temperature of the storage room plays a very significant rôle in determining the rate and extent of deterioration. The present study is not concerned with the effects of storage at various temperatures, all the material having been held at temperatures ranging between 20° and 30° C.

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DEHULLING BARLEY SEED WITH SULPHURIC ACID TO INDUCE INFECTION WITH COVERED SMUT[1]

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INTRODUCTION

Since an easy and certain method of obtaining infection is necessary to a successful study of resistance to covered smut of barley, *Ustilago hordei* (Pers.) Kell. and Sw., in hybrid populations of any considerable size, the lack of such a method has delayed investigations to determine the resistance of barley to this disease. To find a simpler and surer method of obtaining high percentages of infection in barley, a series of experiments was begun in the fall of 1925 in the greenhouse of the California Agricultural Experiment Station.

REVIEW OF LITERATURE

Tisdale[3] obtained a much more abundant infection with covered smut of barley when the seeds were dehulled than when they were left in the natural or hulled state. The hulls were removed by hand with a scalpel or sharp knife, which is at best a slow and tedious process. Seed of Tennessee Winter barley dehulled and inoculated produced 85 per cent of diseased plants, compared with about 4 per cent produced by the inoculated hulled or natural seeds. Similar results were obtained with 10 other varieties. One variety, Nakano Wase, showed no smut even when the seeds were dehulled. Tisdale suggests the desirability of a more efficient method of dehulling the seeds or a simpler means of obtaining satisfactory infection.

Abundant infections with covered smut of barley were obtained by Faris[4] with hulled seed in experiments where the temperature, moisture, and P_H of the soil were under control during the period of infection. Satisfactory percentages of infection occurred over wide ranges of soil temperature and acidity and at moistures well within those frequently existing when barley is sown in the field. Faris also obtained high percentages of infection in Hannchen and Nepal barleys under ordinary field conditions when the seeds were inoculated with spores collected from these respective varieties. He points out that for these two varieties, at least, the one requirement necessary to produce high infection was to use smut collected from the variety on which it was to be tested.

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[2] Throughout the investigation here reported the writer received many suggestions from W. W. Mackie, Division of Agronomy, University of California, and from various members of the staff of the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and he wishes to acknowledge the assistance thus rendered.

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The existing knowledge of the resistance of barley to covered smut is comparatively meager and fragmentary. Varietal tests conducted at the California Agricultural Experiment Station by the writer under the immediate direction of Mackie [5] resulted in almost no infection. Other investigators [5] have experienced the same disappointment.

EXPERIMENTAL METHODS

Experiments in the inoculation of Coast (C. I. 690) barley with covered smut were begun in the fall of 1925. Spores for these experiments were collected from the same variety at Davis, Calif., during the previous summer. Several experiments were tried, in which the seeds were germinated in moist chambers on greenhouse soil, on sand, and on blotter paper moistened with various nutrient solutions. That the spores would germinate readily in the solutions used had been previously determined. In one series, dry spores were dusted on the seeds at the time they were placed in the moist chambers and at daily intervals thereafter until the plumulas were 1 cm. long, when the seedlings were transferred to pots in the greenhouse. In a similar series the seeds were sprayed with a suspension of germinating spores. Practically negative results were obtained in both series. An examination from time to time showed that the spores appeared to be growing normally. Controls of hulled seeds and seeds dehulled by hand were included in each set. The hulled seeds almost invariably produced smut-free plants, whereas the dehulled seeds in soil cultures produced from 20 to 55 per cent of diseased plants. It therefore seemed desirable to look for a simpler method of dehulling the seeds.

The possibility of a chemical method of dehulling the seed was attractive. Sulphuric acid was chosen as a possible reagent because of certain of its properties. It has long been used to treat certain hard seeds in order to promote prompt germination.[6] As early as 1907 Brown [7] found that the seed coat of barley was remarkably resistant to the action of sulphuric acid. He was interested in the semipermeability of membranes, and used a blue-seeded barley for his experiments because the pigment in the aleurone layer is turned red by acids. When the seed coat was intact, soaking in an acid solution of 36 per cent concentration for 44 hours caused no penetration of the seed coat.

EXPERIMENTS WITH CONCENTRATED SULPHURIC ACID

Preliminary experiments by the writer indicated that barley seeds may germinate after being immersed in concentrated sulphuric acid for one hour. However, the young seedlings produced from seeds treated for 20 minutes showed signs of injury. The seeds usually were immersed in a quantity of acid from two to three times the volume of the seeds. The excess acid was drained off before washing by pouring

[5] STAKMAN, E. C. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1921. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul., sup. 21: 208–209, illus. 1922. [Mimeographed.]

[6] LOVE, H. H., and LEIGHTY, C. E. GERMINATION OF SEED AS AFFECTED BY SULFURIC ACID TREATMENT. N. Y. Cornell Agr. Expt. Sta. Bul. 312, p. 293–336, illus. 1912.

[7] BROWN, A. J. ON THE EXISTENCE OF A SEMI-PERMEABLE MEMBRANE ENCLOSING THE SEEDS OF SOME OF THE GRAMINEAE. Ann. Bot. [London] 21: 79–87. 1907.

——— THE SELECTIVE PERMEABILITY OF THE COVERINGS OF THE SEEDS OF HORDEUM VULGARE. Roy. Soc. [London] Proc. (B) 81: 82–93. 1909.

the seeds into a small wire basket which had been coated with paraffin to resist the action of the acid. The basket of treated seeds was plunged into a sink full of cold water and then placed for 10 minutes under a faucet fitted with a rose or shower nozzle. Finally, the excess water was removed with a towel, and the seeds were spread out in the laboratory to dry. Commercial sulphuric acid proved to be nearly as effective in removing the barley hull as C. P. acid and is considerably cheaper. The commercial acid therefore was used in all of these experiments.

Hand-threshed seed of Tennessee Winter (C. I. 257) barley was used for the acid experiments. The smut was from Coast (C. I. 690), as in the former experiment. The seeds were thoroughly dusted with an excess of spores. Twenty-one seeds from each treatment were placed in 8-inch pots and covered uniformly to a depth of 1 inch. The pots were left in the head house, where the temperature was favorable for infection. The temperature ranged from 7.8° to 17.7° C. When the seedlings were 2 or 3 inches high the pots were moved into the greenhouse. The percentages of infection obtained after dehulling barley seeds with concentrated sulphuric acid are reported in Table 1.

TABLE 1.—*Infection with covered smut in Tennessee Winter barley grown from seeds treated with concentrated sulphuric acid and seeded in the greenhouse on February 15, 1926, at Berkeley, Calif.*

[TWENTY-ONE SEEDS USED FOR EACH TREATMENT]

Treatment	Plants produced		Plants smutted	
	Number	Per cent	Number	Per cent
None	21	100.0	0	0
Seeds dehulled by hand	19	90.5	4	21.1
Seeds treated with concentrated H_2SO_4 10 minutes	19	90.5	4	21.1
Seeds treated with concentrated H_2SO_4 20 minutes	5	23.8	2	40.0
Seeds treated with concentrated H_2SO_4 30 minutes	1	4.8	1	100.0

There was no smut on any plants produced by hulled seeds, whereas 21 per cent of the plants produced by dehulled seeds were smutted. The 10-minute acid treatment resulted in a satisfactory stand of plants which showed the same percentage smutted as the stand from the dehulled seeds. A very thin hull remained after this treatment. It was so thin that the plumules broke through the hull instead of pushing out between the hull and the kernel, as is the rule in hulled seeds. Treatment with acid for 20 or 30 minutes reduced the stand too much to be of practical value.

EXPERIMENTS WITH DILUTED SULPHURIC ACID

FIRST EXPERIMENT

Another series of experiments was started with seeds treated with diluted sulphuric acid. A concentration of acid was sought which would digest the entire hull without injury to the seeds. A scale of acid concentrations ranging from 40 to 90 per cent was employed. The time of treatment was somewhat arbitrary in each case, except that a few seeds were removed from time to time and the hulls and

seed coats examined. Forty-five seeds from each treatment were placed in 12-inch pots and covered uniformly to a depth of 1 inch. The pots again were left in the head house until the plants had emerged. The temperature ranged from 10° to 23.3° C. The percentages of smut from the lots of seeds treated with the various dilutions of acid are recorded in Table 2.

TABLE 2.—*Infection with covered smut in Tennessee Winter barley grown from seed treated with various concentrations of sulphuric acid and seeded in the greenhouse on March 11, 1926, at Berkeley, Calif.*

[FORTY-FIVE SEEDS USED FOR EACH TREATMENT]

Lot No.	Concentration of acid	Duration of treatment	Degree of dehulling	Plants produced		Plants smutted	
				Number	Per cent	Number	Per cent
	P. ct.	*H. m.*					
1	40	6 0	Thin hull remaining over entire seed	38	84.4	8	21.1
2	40	7 0	Thin hull remaining over germ half of seed	39	86.7	10	25.6
3	40	8 0	Thin hull remaining over germ	43	95.6	18	41.9
4	50	5 0	Thin hull remaining over germ half of seed	39	86.7	17	43.6
5	50	6 0	Thin hull remaining over germ	40	88.9	15	37.5
6	50	7 0	Mostly dehulled; thin hull remaining over germ of a few	35	77.8	4	11.4
7	60	1 30	Thin hull remaining intact	45	100.0	6	13.3
8	60	2 15	Thin hull remaining over germ	37	82.2	5	13.5
9	60	3 0	Mostly dehulled	21	46.7	4	19.0
10	70	30	Thin hull remaining over germ half of seed	43	95.6	7	16.3
11	70	45	Mostly dehulled	33	73.3	9	27.3
12	70	1 0	do	12	26.7	6	50.0
13	80	25	Very thin hull remaining over entire seed	42	93.3	12	28.6
14	80	40	Thin hull remaining over germ	26	57.8	11	42.3
15	80	50	Mostly dehulled	3	6.7	1	33.3
16	90	15	Thin hull remaining over entire seed	41	91.1	11	26.8
17	90	25	Very thin hull remaining on most	30	66.7	14	46.6
18	90	35	Thin hull remaining over germ	5	11.1	4	80.0
19	Control.	.....	Dehulled by hand	41	91.1	11	26.8
20	..do..	.....	Hulled	42	93.3	0	0

Obviously it is difficult to describe the degree of dehulling in a few words. Three general conditions of dehulling existed, with all the intermediate stages present. After some of the shorter treatments a thin hull still persisted over the entire kernel, a condition similar to that described after the 10-minute concentrated-acid treatment. After treatments of intermediate severity, the hull remained only over the germ. The hull is considerably thicker over the germ and naturally more time is required for the acid to digest this portion. After the more severe treatments the hull was found to be entirely removed from most of the seeds by the acid. Concentration of 80 and 90 per cent acid left the seed coats with a corroded appearance.

Stands of less than 30 plants are not considered desirable and may be dropped from further consideration. Plants from 8 of the 13 remaining treatments contained percentages of smut as high as or higher than those from the seed dehulled by hand. Plants from the hulled lot, on the other hand, were free from smut. The dilute solutions of acid appear to be more desirable than the concentrated solutions, both because of abundant smut infection and because of

lack of seed injury. Just why the 60 per cent acid treatment was followed by such uniformly low percentage of smut can not be explained at this time. An examination of some of the seeds from this treatment indicated that the acid was not as completely removed as after most of the other treatments. The degree of dehulling was as complete as was the case with several other treatments where higher infection occurred. There seems to be considerable variation in smut infection after apparently equal degrees of dehulling. Considerable variation may be expected, however, where such small numbers of plants are used.

TABLE 3.—*Infection with covered smut in Tennessee Winter barley grown from seed treated with various concentrations of sulphuric acid and seeded in the greenhouse on November 26, 1926, at Berkeley, Calif.*

[FORTY-FIVE SEEDS USED FOR EACH TREATMENT]

Lot No.	Concentration of acid	Duration of treatment	Degree of dehulling	Plants produced		Plants smutted	
				Number	Per cent	Number	Per cent
	P. ct.	*H. m.*					
1	30	24 0	All dehulled	14	31.1	14	100.0
2	40	6 0	Hull remaining on few germ ends	33	73.3	29	87.9
3		7 0	All dehulled	27	60.0	24	88.9
4		8 0	do	29	64.4	22	75.9
5	50	4 0	do	26	57.8	23	88.5
6		5 0	do	16	35.6	13	81.2
7		6 0	do	10	22.2	10	100.0
8	60	1 30	Hull remaining over germ end	34	75.6	30	88.2
9		2 0	Hull remaining over germ end of half of seeds	16	35.6	14	87.5
10		2 30	All dehulled	2	4.4	2	100.0
11	70	30	Hull remaining over germ half of kernel	40	88.9	29	72.5
12		40	Hull remaining over germ end	35	77.8	31	88.6
13		50	Very thin hull remaining over germ end	22	48.9	20	90.9
14	80	25	Hull remaining over germ end	22	48.9	16	72.7
15		35	Thin hull remaining over germ end	3	6.7	3	100.0
16		45	Most kernels dehulled	1	2.2	1	100.0
17	90	15	Very thin hull remaining	10	22.2	7	70.0
18		20	Hull remaining over germ end	10	22.2	7	70.0
19		25	Very thin hull remaining over germ end	5	11.1	4	80.0
20	Concentrated	5	Thin hull remaining intact	38	84.4	33	86.9
21		10	Very thin hull remaining intact	25	55.6	19	76.0
22		15	Hull remaining over germ end	3	6.7	3	100.0
23	Control	------	Dehulled by hand	22	48.9	16	72.7
24	do	------	Hulled	45	100.0	12	26.7

SECOND EXPERIMENT

On November 26, 1926, another series of experiments was started in the greenhouse, in which were used dilutions of sulphuric acid ranging from 30 per cent to concentrated. Seed of Tennessee Winter (C. I. 257) barley was again employed. Instead of using smut from a single variety, as in the former experiment, the inoculum consisted of a composite sample from the following varieties: Tennessee Winter (Calif. 2272 and C. I. 257), Coast (C. I. 690), Atlas, Club Mariout, Smyrna, Hero Selection (C. I. 1286–1), Vaughn, and Flynn. The seeds were washed for 20 minutes instead of 10, as in previous experi-

ments. Forty-five seeds from each treatment were placed in 12-inch pots and covered uniformly to a depth of 1 inch. During the period of infection the pots were left in the head house, where the temperature ranged from 12.2° to 16.7° C. When the seedlings were about 2 inches high the pots were moved into the greenhouse. The results from this experiment are given in Table 3.

The percentages of smut are much higher after all of these treatments than after those of the preceding experiment. Whether the higher infections were due to more favorable environmental conditions or to the inoculum used is not at present known. The soil and the soil moisture were the same. The temperature in the earlier experiment ranged from 10° to 23.3° C. as compared with 12.2° to 16.7° in the later experiment. The lower maximum temperatures were due to the prevalence of cloudy weather.

The number of plants developing after most treatments was considerably smaller in the later than in the earlier experiment. Even from the lot of hand-dehulled seeds there were only 22 plants in the second experiment as compared with 41 in the first. It is interesting to note that in the second experiment there were 45 plants, or a 100 per cent stand, where the seeds were not dehulled. If 30 or more plants are considered a satisfactory stand, five of the acid treatments were followed by satisfactory stands while hand-dehulled seed was not. Eleven of the acid treatments were followed by stands equal to or better than that from the hand-dehulled seeds. The percentage of smut in the plants from the acid-treated seeds was as high as or higher than in those from the hand-dehulled seeds. The plants from the hulled seeds had a slightly higher percentage of smut in this experiment than those from the hand-dehulled seeds in the previous experiment (Table 2). However, the dehulled seeds allowed much better infections in each experiment than did the hulled seeds.

The treatments with diluted acid caused more complete dehulling in the first experiment than in the second. The reason for this is not apparent at present. The data thus far indicate that it is not necessary to dehull the seed completely in order to induce satisfactory smut infections. In fact, the best stands, accompanied with satisfactory smut infection, resulted where a thin hull remained over the germ end of the seed or over the entire kernel.

FIELD EXPERIMENT WITH ACID-TREATED SEED

A very limited experiment with acid-treated seed was made in the field at Davis, Calif., to determine the effectiveness of this method of infection under field conditions. Coast (C. I. 690) barley, inoculated with spores of covered smut from the same variety, was used in this experiment. Duplicate rod rows from each treatment were seeded. Seventy-five seeds were sown in each row and covered as uniformly as possible. The field results are recorded in Table 4.

The plants from the hulled seeds produced 10.3 per cent of smut, which is only about half that produced by those receiving the 50 per cent acid treatment. Seeds treated with concentrated acid for 10 minutes produced results equal to those from the hand-dehulled seeds. Row 8 may have been at a slight disadvantage, as it was located very near an irrigation levee. The acid-treated seeds at least compare favorably with the hand-dehulled seeds both from the standpoint of stand and of smut produced.

TABLE 4.—*Infection with covered smut in Coast barley grown from seeds treated with sulphuric acid and seeded in the field on March 1, 1926, at Davis, Calif.*

[SEVENTY-FIVE SEEDS IN EACH ROW]

Row No.	Treatment	Plants produced		Plants smutted		Average percentage of smut
		Number	Per cent	Number	Per cent	
1	None	65	86.7	6	9.3	
2	do	62	82.7	7	11.3	10.3
3	Dehulled by hand	60	80.0	15	25.0	
4	do	40	53.3	14	35.0	30.0
5	Concentrated H_2SO_4, 10 minutes	53	70.7	20	37.7	
6	do	67	89.3	18	27.0	32.3
7	50 per cent H_2SO_4, 10 minutes	55	73.3	13	23.6	
8	do	35	46.7	7	20.0	21.8

DISCUSSION

Hulled seeds produced plants showing only low infections with covered smut, as has been the case in previous experiments at this station. In 1919–20 W. W. Mackie [8] and the writer made duplicate rod-row seedings of 570 varieties and strains of barley at both Davis and Salinas, Calif. Although the seeds were heavily inoculated with viable spores of covered smut, practically no smut occurred. The following year about 100 varieties were sown at each of these stations with similar results. In no case did the smut infection exceed 5 per cent, although from 10 to 15 per cent of smut occurred in a field seeded by a farmer with untreated seed on the same day on land adjacent to one of the stations.

Dehulled seeds constantly produced fairly satisfactory infections. Only in the last experiment were the amounts of smut of the same magnitude as those obtained by Tisdale.[9] As already pointed out, Faris [10] was able to get a very high percentage of infection with hulled seed where the temperature, moisture, and P_H were controlled. However, these factors are not easily controlled where extensive sowings are necessary. Unsatisfactory infections (Table 4) occurred with hulled seed even when smut from the variety suggested by Faris [11] was used. On the other hand the writer obtained fairly satisfactory infections by dehulling the seeds even under conditions where no smut was produced when hulled seeds were used. Under the conditions of these experiments, plants grown from seeds treated with sulphuric acid showed percentages of infection which compared favorably with those of plants grown from hand-dehulled seeds. With proper facilities for washing, many lots of seeds may be treated with sulphuric acid in a day. Seeds may be washed very easily at the rate of 12 lots an hour. Considerable saving in time and money is gained over the hand-dehulling method.

Data are not numerous enough to justify a positive opinion as to the most satisfactory strength of acid and the proper duration of treatment. Additional experiments have been started in the field

[8] STAKMAN, E. C. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1921. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul., sup. 21, p. 139–254, illus. 1922. [Mimeographed.]
[9] TISDALE, W. H. Op. cit.
[10] FARIS, J. A. Op. cit.
[11] FARIS, J. A. FACTORS INFLUENCING INFECTION OF HORDEUM SATIVUM BY USTILAGO HORDEI. Amer. Jour. Bot. 11: 209. 1924.

at Davis and in the greenhouse at Berkeley, which should yield results indicating the most satisfactory treatment. Because the thickness of the hulls in barley varies with the variety, it may be rather difficult to establish a standard treatment which will be equally satisfactory for all varieties. Preliminary experiments with 10 varieties of barley, to be hereafter reported, seem to indicate that this is not an important matter.

Injury to the seed coat in threshing must be avoided if the seeds are to be treated with acid. The barleys used in these experiments were hand threshed, but preliminary experiments not reported in this paper indicate that seed threshed with a small nursery thresher are practically free from seed-coat injury and may be treated with sulphuric acid without damage from this source.

Covered smut of barley may be controlled by the use of fungicides, but, because this disease is rather sporadic in its occurrence in California, seed treatment is not regularly practiced by many farmers, and consequently considerable losses occur from time to time. Because the acid method of inducing smut infection is comparatively simple and certain, varieties of barley in large numbers may not be tested for resistance to covered smut. Any resistant varieties which are isolated may be used in studying the inheritance of resistance to this disease. Finally, the breeding of resistant varieties suitable for the various barley-growing regions offers a promising means of eventually eliminating covered smut.

SUMMARY

Experiments were begun in the greenhouse at Berkeley, Calif., in the fall of 1925, to obtain an easier method of satisfactorily infecting barley with covered smut.

In each experiment barley seeds dehulled by hand before inoculation produced plants with percentages of smut much higher than plants from the natural or hulled seeds.

Seeds subjected to several different treatments with sulphuric acid produced stands equal to those from the hand-dehulled seed, and the resulting plants contained similar percentages of smut.

A very limited field seeding indicates that the acid treatments will give results in the field similar to those obtained in the greenhouse.

Data are not numerous enough at present to indicate the most desirable strength of acid or the proper duration of treatment. Additional experiments in the greenhouse at Berkeley and in the field at Davis have been begun to determine these points.

Experiments to date indicate that it is not necessary to remove the barley hull completely in order to induce satisfactory infection with covered smut. The best stands accompanied by satisfactory infection were obtained when a thin hull remained over the entire kernel or at least over the germ end.

The acid treatment herein described will so simplify the method of inducing covered smut in barley that varieties may be tested in large numbers for resistance to this disease, and the segregation of smut-resistant forms in hybrid generations may be studied.

THE GROWTH OF BACILLUS RADICICOLA ON ARTIFICIAL MEDIA CONTAINING VARIOUS PLANT EXTRACTS[1]

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INTRODUCTION

A review of the voluminous literature on legume-nodule bacteria brings out the fact that although the organisms will live and retain their vitality on a large variety of liquid artificial media, they seldom produce thick films and large masses of viscous surface growths except in the presence of certain miscellaneous nitrogen-containing mixtures, such as yeast extract or legume extracts. On a simple synthetic inorganic medium containing, in addition, an available source of energy, growth has usually been reported as small. If nitrates are added to this synthetic medium, the liquid culture usually becomes more cloudy and the gum production may show an increase, but if the sugar used is pure, heavy viscous film growths are seldom if ever obtained. Dawson (*1*)[3] describes such growths as thick, zoogloealike films. They commonly form from 7 to 10 days after inoculation and may settle to the bottom of the flask a few days later. This paper presents the results of qualitative studies of the effect of plant extracts upon the production of such viscous film growths.

Before proceeding to a discussion of the experimental work, it is well to emphasize that these studies were not undertaken with the idea of developing a more satisfactory medium than those now in common use; neither does the work deal with physiological efficiency, as judged by inoculating power. These subjects have been thoroughly considered by many investigators. Neumann (*5*) studied about 70 kinds of media, including plant extracts and soil extract. Zipfel (*8*) observed the growth in various media over a range of temperatures and at different H-ion concentrations. Temple (*7*) particularly refers to the beneficial effects of adding a small amount of leguminous material to sterile soil for the culture of the organisms. Prucha (*6*) at the Cornell station has contributed to our information on various phases of culture work. Fred and his associates at Wisconsin have done some very excellent work. One of the pioneers in this line of investigation was Moore (*3*, *4*). Later work from the same laboratory by Kellerman and his associates has added greatly to our knowledge of methods for handling cultures for commercial use. Dozens of other references might be given if space permitted. From all of these studies it is apparent that such media as soil extract, wood ash, and yeast water are excellent for routine work. The writer uses soil-extract media almost exclusively for maintaining stock cultures in an active state and considers them excellent.

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[2] The writer wishes to thank K. F. Kellerman, L. T. Leonard, and N. R. Smith of the Bureau of Plant Industry, United States Department of Agriculture, for their cooperation in supplying most of the cultures used in this work, and for their helpful suggestions.

[3] Reference is made by number (italic) to "Literature cited," p. 923.

The purpose of this investigation was to determine some of the conditions which favor the production of zoogloealike film growths; the types of plant extracts and other materials which cause them; and finally, if possible, the particular constituent, if any, which is responsible for such unusually vigorous cell multiplication. It was desired to know if the stimulating agent in the extracts is a food or a true stimulant. If a stimulant or a nonnitrogenous food, it was hoped that its separation from plant extracts and its addition to synthetic nitrogen-free media might make conditions for the growth of the organism sufficiently ideal to favor nitrogen fixation on a scale comparable to that obtained with Azotobacter. The present paper is of a preliminary nature and deals only with the growth-promoting effects of various plant extracts.

MEDIUM USED

In beginning these studies it was necessary to select a suitable combination of inorganic elements, together with an available source of energy, to serve as a basis for all media used throughout the investigations. This mixture should supply as much of all the common elements as is needed for use in heavy growth of the organism, except, of course, those elements which are intended for variables. Plant extracts or other materials, added to this combination, would give more inorganic elements and sugars, but this could not be avoided.

The final selection of the combination of inorganic elements was based both upon the results reported in the literature and upon tests of about 100 combinations made in this laboratory. Space does not permit a detailed description of these experiments but, in brief, it may be stated that *Bacillus radicicola* responds particularly to phosphorus, and in the case of some strains, to calcium. A relatively small quantity of the other necessary inorganic elements, other than nitrogen, is sufficient. The combination finally chosen had the following composition, expressed in grams: K_2HPO_4, 0.2; $MgSO_4$, 0.2; NaCl, 0.2; $CaSO_4$, 0.1; $FeCl_3$, 0.005; $CaCO_3$, 1.0; and H_2O, 1000.0. This medium, with a suitable source of energy, supplied all of the 10 elements, other than nitrogen, which are considered as most needed by plants; it is wholly synthetic, and furnishes an adequate but not excessive supply of the ingredients.

Several experiments were made to determine the best source of energy for legume bacteria. Of 30 sources tested, 10 were about equally good for a large number of strains of the organism, but some were particularly good for certain strains. Sucrose has been used to the greatest extent by other workers, but at this laboratory it seemed to be no better than several other sugars. It is well to emphasize the fact that the strains differ. The organism used to the greatest extent in these experiments, a red clover culture, showed a slight preference for dextrose, and for this reason this sugar was used in all the plant extracts studied.

No source of nitrogen was included in the media because *Bacillus radicicola* is supposed to fix atmospheric nitrogen. In the first experiments reported below no nitrogen was added to the culture medium except that supplied in the plant extracts. Further reference to the part which nitrogen plays in the results will be made in subsequent paragraphs.

ESTIMATION OF GROWTH

Unfortunately, there is no satisfactory quantitative method for measuring the amount of growth in culture flasks. This statement may seem strange at first, and needs explanation. This study deals with a particular type of growth which can be seen, but which is not easy to measure. The following three possibilities suggested themselves: (1) Obtain the dry weight of the bacterial cells, (2) determine numbers, and (3) separate out the growth and determine the nitrogen.

The determination of dry weight was made in a few instances, but this method was entirely unsatisfactory, especially in the presence of plant extracts. It is very difficult to filter off the growth because of the presence of the bacterial gum, which becomes a portion of the dry weight unless it is removed. But its removal is practically an impossibility, because it is formed as an effluent cell wall of the organism. The presence of $CaCO_3$ and miscellaneous plant constituents, some of which may be precipitated by autoclaving or bacterial growth, added to the difficulties.

Bacterial numbers might have been determined, but the writer considers this determination practically worthless for the problem in hand, as well as for most similar problems. Apparently this is the general consensus of opinion among bacteriologists, judging from the discussions at the recent meetings of the First International Congress of Soil Science at Washington. Particularly is it true in the present instance, because the thing to be estimated is a particular type of growth. The heavy viscous films of bacteria on plant-extract media consist largely of masses of giant cells, gonidia, and budding cells held together very closely by the sticky gum. No ordinary system of dilutions would satisfactorily separate the bacteria present in budding clumps, and hundreds or even thousands might form the nucleus of a single colony on a plate. Assuming that the cells could be separated, the method is still unsatisfactory, because the results obtained would depend wholly on the type of the organism predominating on any particular day. The life cycle, as shown by Löhnis (*2*), commonly passes within a few days from motile rods (from 1 to 2 microns in length) to giant cells (from 5 to 30 microns) and then to gonidia (0.3 micron). In the case of plant-extract cultures we may add budding clumps as a common occurrence. By plate counts a gonidium 0.3 micron in diameter would be as important as a gonidangium 30 microns long.

A chemical method of estimating the mass of growth was developed. This consisted in precipitating the bacterial gum, which carries with it the organisms, by the addition of a suitable reagent. Basic lead acetate seemed most effective, although alcohol and various other agents may be used. The precipitate was washed thorougly and the nitrogen content determined. The method was not satisfactory where plant extracts and other miscellaneous organic materials containing nitrogen were present. In other cases it gave fair results.

In view of the lack of a satisfactory quantitative method, it was decided to judge the growth by the eye only. This gave a better qualitative picture of the abundance of the type of growth under consideration than might be supposed, particularly for the red-clover

strain of the nodule bacteria used. For the purpose of these investigations qualitative results were satisfactory and quickly obtained.

METHOD OF STERILIZATION OF PLANT EXTRACTS

In beginning these experiments, the logical plant extract to use in obtaining some of the preliminary data was, of course, the juice from red clover, since the organism used was from the nodules of the roots of that plant. Furthermore, other investigators have repeatedly shown that clover, as well as other legume extracts, greatly stimulates the nodule organism.

Four hundred grams (green weight) of red clover plants, including tops, roots, and nodules, were ground in a meat chopper. To the macerated plants was added 300 c. c. of cold water, and after standing about two hours the mixture was filtered. To one series of 250 c. c. Erlenmeyer flasks containing the inorganic ingredients and dextrose were added in duplicate, aliquot parts of the clover extract corresponding to 3, 6, 12, 30, and 60 gm. of the original clover plant per 100 c. c. of medium. These flasks were plugged with cotton and sterilized in the autoclave at 15 pounds pressure for 20 minutes. The remainder of the original extract was first sterilized by passing it through a sterilized Chamberland-Pasteur filter. After incubation for a week to make certain of sterility, aliquot parts of this extract were then transferred under sterile conditions to flasks containing the sterilized salt-sugar medium already described. Inoculations were made with an active pure culture of the red clover organism and growth was allowed to take place at 26° C. Observations, made at intervals, showed no appreciable difference in growth in corresponding flasks sterilized with and without heat. Growth was good in both cases, and the more clover extract present the heavier the surface film produced, with the exception of the flask receiving the most extract. In this flask early growth was slightly retarded. Growth was exceptionally heavy in this flask, however, by the end of a week. Analyses for total nitrogen by the Kjeldahl method showed no fixation.

STUDIES WITH VARIOUS LEGUME EXTRACTS

In order to determine whether the extract made from any given leguminous plant is particularly stimulating for the strain of organisms which normally grows in the nodules of that plant, several additional experiments were made. Included in these tests were some comparisons of the effect of extracts made from the tops, roots, and in some cases from the seeds of a given plant. Incubation was for a period of three weeks at 26° C., and observations were made at frequent intervals. Many of the plant extracts were tested several times under slightly different conditions. The concentration was varied and different batches of the extract were used. It seems scarcely worth while to go into detail regarding all of these separate experiments, but in Table 1 some of the observations are given which particularly show the relation of the concentration of the extract to the results observed. It should be remembered that no nitrogen other than that contained in the extracts was added to any of the flasks.

TABLE 1.—*Growth of red clover nodule bacteria on media containing various legume extracts*

Source of extract	Grams of green plant material extracted per 100 c. c. of medium	Condition of growth *
No plant extract	0	x
Red clover tops and roots	15	xxxx
Red clover tops	2	xx
Do	5	xx
Do	8	x
Do	15	0
Do	30	0
Red clover roots	2	xxxx
Do	5	xxxxx
Do	8	xxxxx
Do	15	xxxxx
Sweet clover tops	2	xx
Do	5	xx
Do	8	xx
Do	15	0
Do	30	0
Sweet clover roots	2	xx
Do	5	xxx
Do	8	xxxxx
Do	15	xxxxx
Do	30	xxx
White clover tops	4	0
Do	12	0
Do	16	0
Navy bean seeds	.4	xx
Do	.7	xxx
Do	1.8	xxx
Do	3.6	xxxx
Do	7.2	xxxx
Do	10	xxxx

* 0=no growth; x=slight growth; xx=fair growth; xxx=medium growth; xxxx=good growth; xxxxx=very good growth.

The data in Table 1 indicate very definitely that the red clover organism does not show a greater response to red clover extract than to certain other legume extracts, such as those from sweet clover roots or from navy bean seed. Another point of considerable significance is the marked difference between the extracts of the tops and the roots of leguminous plants. The extracts of both red clover and sweet clover tops produced slight stimulations at the lower concentrations, but prevented all growth at the 15 gm. and 30 gm. rates. Even at the lowest concentration of 4 gm., the extract of white clover tops prevented growth. The appearance of the cultures suggested that poor growth was probably due to the presence of a toxic material. Several chemical tests were made in order to determine definitely if such were the case, and it was found that an extract from red clover tops, after precipitation with alcohol and removal of the precipitate, was very active in increasing bacterial growth. No attempt was made to identify the toxic material. Analyses of a portion of the cultures listed in Table 1 showed no gains in nitrogen.

STUDIES WITH VARIOUS NONLEGUME EXTRACTS

In order to determine if juices from nonlegume plants also favor luxuriant growth of *Bacillus radicicola*, several vegetables and other plants were used and tested in the same way without the addition of any nitrogenous compounds other than those in the plant juice. Extractions were made by grinding the materials, adding a little

$CaCO_3$, boiling the mixture for a few minutes, and then filtering it. The results are shown in Table 2.

TABLE 2.—*Growth of red clover nodule bacteria on media containing various nonlegume extracts*

Source of extract	Grams of green plant material extracted per 100 c. c. of medium	Condition of growth [a]
No plant extract	0	x
Banana	2	x
Do	5	x
Do	8	xxx
Do	15	0
Do	30	0
Beet roots	2	xx
Do	5	xx
Do	8	xx
Do	15	xx
Do	30	xx
Do	50	xx
Blue grass tops	6	xxx
Do	12	xxxx
Do	24	xxxxx
Cabbage	2	xx
Do	5	xxx
Do	8	xxx
Do	15	xxxxx
Do	30	0
Carrot	11	xxxx
Do	22	xxxxx
Green corn tops	10	xxxx
Do	20	xxxxx
Do	60	xxxxx
Lettuce	2	xx
Do	5	xx
Do	8	xxx
Do	15	xxxx
Do	30	xxxxx
Orchard grass tops	2	xx
Do	5	0
Do	8	0
Do	15	0
Do	30	0
Orchard grass roots	2	x
Do	5	xx
Do	8	xxx
Do	15	xxxx
Parsnip	10	xxx
Do	20	xxxx
Potato	9	xx
Do	18	xxx
Do	45	xxxx
Spinach	8	x
Do	1.5	x
Do	4	xx
Do	8	xxxx
Do	12	xxxxx
Do	16	xx
Sweet potato	3	xx
Do	6	xx
Do	15	x
Do	21	0
Do	42	0
Turnip	11	x
Do	22	xx

[a] 0=No growth; x=slight growth; xx=fair growth; xxx=medium growth; xxxx=good growth; xxxxx=very good growth.

It will be observed that the extracts from a large number of plants favor the growth of legume bacteria, there being little difference between many nonlegumes and legumes. When used in the optimum concentrations, extracts of orchard-grass roots, lettuce, cabbage, carrot roots, spinach, corn tops, and bluegrass produced growths

comparable to those produced by legume-root extracts. Banana, potatoes, and parsnip extracts also produced good growths, but less than the above-named plants. Results obtained from extracts from orchard grass tops, sweet potato, and turnip were the poorest. Here the same observations may be made as are indicated in Table 1, namely, that some extracts prevent growth if used in too high a concentration, probably because of the presence of toxic compounds. Here again analyses failed to show any nitrogen fixation by *Bacillus radicicola*.

When this work was undertaken the writer expected to find that extracts of leguminous plants would produce much better media than those of nonlegumes, if reinforced with sugar and the necessary inorganic salts; but this did not prove to be true. If the activity were limited to plants which produce nodules, then it would be logical to infer that the stimulant or food plays an important rôle in symbiosis. The experiments reported here probably have little bearing on symbiosis.

IMPORTANCE OF NITROGEN

Since the above experiments had failed to demonstrate any powers of nitrogen fixation by the red-clover organism under the conditions of the experiment, it seemed very desirable to compare the growths resulting from several plant extracts with an available source of nitrogen added. Another series of experiments was, therefore, carried out, giving a direct comparison between the growths in the presence and in the absence of a nitrogenous salt. One hundred milligrams of KNO_3 was used per 100 c. c. of medium, because several tests, which can not be included here, had shown this to be a very satisfactory source of nitrogen. Because of the large number of extracts tested simultaneously, only two concentrations could be used; but with the previous work to serve as a guide, it was not difficult to select concentrations that would bring out the salient points.

All extracts were prepared by grinding 100 gm. of the plant material, adding about 5 gm. of $CaCO_3$ and 200 c. c. of water, and autoclaving at 15 pounds pressure for 20 minutes. After filtration the extracts were reduced to dryness on a steam bath, again diluted, filtered, and made up to 500 c. c. Extreme difficulty was experienced in the filtration of some of the extracts, but by using both the centrifuge and suction filter, where necessary, a reasonably clear extract was obtained in all cases. Two extracts were made in the case of yeast, one of a good commercial yeast in cake form and the other of yeast cells grown on a synthetic medium. Table 3 gives the comparative growths, as determined by observation, for each of the extracts under the various conditions of the tests. In considering these results it should be remembered that the figures in the table showing the quantity of extract used are based on the green weights of the plants. If the tests had been on the dry basis the relative activity would have been quite different. These experiments were not planned, however, as quantitative tests, but merely to gain a general idea of the stimulating properties of various extracts used in the presence and in the absence of an available nitrogenous salt.

TABLE 3.—*Comparative tests of various plant extracts without and with added nitrogen*

Source of extract	Grams of green plant material extracted per 100 c. c. of medium	Condition of growth without KNO_3[1]	Condition of growth with KNO_3 [1]
No plant extract	0	x	x
Alfalfa seeds	2	xxx	xxxx
Do	10	xxx	xxxxx
Apple	2	x	x
Do	10	x	x
Banana	2	x	x
Do	10	xxx	xxxx
Beet roots	2	x	x
Do	10	x	xx
Cabbage	2	xx	xx
Do	10	xxx	xxxx
Carrot	2	x	x
Do	10	xxx	xxxxx
Carrot tops	2	x	x
Do	10	0	0
Clover seeds	2	xxx	xxxx
Do	10	xxx	xxxxx
Corn meal	2	x	xx
Do	10	xx	xxx
Cowpea seeds	2	xxx	xxx
Do	10	xxxxx	xxxxx
Lettuce	2	x	x
Do	10	xxx	xxxxx
Orange	2	xx	xx
Do	10	xxx	xxxx
Parsnip	2	xx	xx
Do	10	xxx	xxxx
Potato	2	xx	xx
Do	10	xxx	xxxx
Soy-bean seeds	2	xx	xxx
Do	10	xxxxx	xxxxx
Spinach	2	xx	xx
Do	10	xxxx	xxxxx
Sweet potato	2	x	x
Do	10	xx	xx
Tomato	2	xx	xx
Do	10	xxxx	xxxxx
Turnips	2	x	x
Do	10	xx	xx
Yeast, cultivated		x	x
Do		x	xx
Yeast, commercial	2	xxx	xxxx
Do	10	xxx	xxx

[1] 0=no growth; x=slight growth; xx=fair growth; xxx=medium growth; xxxx=good growth; xxxxx=very good growth.

From the data in Table 3 it will be observed that the addition of KNO_3 to culture flasks which contained the various plant extracts usually increased the growth and was in no case injurious, regardless of the quantity of nitrogen added. Even the growths in the presence of the extracts of some plant products, like clover seed and alfalfa seed, which contain an abundance of nitrogen, were greater with nitrogen added. This shows the necessity for the addition of a source of nitrogen in all cases where tests such as those reported here are to be made. Table 3 also shows that plants which represent very widely different species contain excellent sources of food for legume bacteria. Orange juice, tomatoes, lettuce, and spinach would show an especially high activity if comparisons were made on a dry basis. The least stimulating extracts were those from beets, sweet potatoes, turnips, apples, and carrot tops. No experiments were made to determine if the low activity of these extracts was due to the presence of toxins, as was

previously found to be true in the case of red clover tops. In these experiments yeast isolated in pure culture from a commercial yeast cake and grown on a synthetic medium failed to show any appreciable synthesis of the bacterial stimulant or food; on the other hand, an extract of the commercial yeast cake was very active. The activity can probably be attributed to the original ingredients used in preparing the commercial product.

SUMMARY

A study of the growth-promoting properties of plant extracts for legume-nodule bacteria is reported. The results given were obtained with a red clover organism, this culture having shown a greater response in preliminary tests than any other strain used. It was found that certain strains of the organism, which ordinarily make a poor growth on a simple sugar-salt medium, produce luxuriant growth in the presence of the juices of certain plants. Extracts were prepared from 26 different plant materials, both legumes and nonlegumes, including vegetables, fruits, and field crops. The extracts from practically all of these increased bacterial growth to some extent, at least when used in the proper concentration; but the activity varied widely for different plants and even for different parts of the same plant. Extracts of legume roots particularly favor the growth of *Bacillus radicicola*. Of the nonlegume extracts, the best were from lettuce, cabbage, carrot, spinach, tomato, green corn tops, bluegrass, and orange. The bacterial food value of any extract of any given species of plant varies widely with different samples, depending largely on the stage of maturity.

These studies have shown very definitely that there is a wide difference in the response of various strains of legume bacteria to plant-extract media. Of 30 strains taken from as many different species of plants, the clover-alfalfa group of organisms gave the largest increases in growth. Many strains will, however, show increasing response to plant extracts after several transfers on the same medium.

A report of experiments to determine the nature of the plant constituents which are primarily responsible for the production of such abundant growths of certain strains of legume-nodule bacteria, when the extracts are added to artificial liquid media, will be given in a subsequent paper.

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RELATION OF TEMPERATURE TO GROWTH OF PENICILLIUM ITALICUM AND P. DIGITATUM AND TO CITRUS FRUIT DECAY PRODUCED BY THESE FUNGI [1]

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INTRODUCTION

Two of the most important decay-producing fungi in citrus fruits are *Penicillium italicum*, which produces blue contact mold, and *P. digitatum*, which produces common green mold. The investigation of these fungi in relation to temperature had for its purpose the gaining of information not only on the rate of growth from day to day on artificial media but also on the rate and percentage of decay produced in orange fruits. The results were found to show interesting differences in rate of decay between the stem end and the stylar end of the fruit.

METHODS

The cultures of the two fungi were grown on glucose-potato agar in Petri dishes 90 cm. in diameter, each dish containing 10 c. c. of the medium. Minute drops of water containing a few spores were placed on this medium at the center of each dish, and the dishes were promptly placed in the temperature chambers. The fluctuations in each chamber were about one-half degree centigrade. The diameter of the circular mycelial mat in each dish was measured at intervals of two days.

The fruits used in the experiments were Valencia oranges from Orange County, Calif., picked during the latter part of August, 1925. They had been washed in the packing house and were prepared by being rinsed with 95 per cent alcohol, injured with uniform clipper cuts approximately one-half centimeter in diameter at both the stem and stylar ends, and inoculated by brushing spores on the injuries. The clippers were dipped in alcohol and flamed before each cut. The spores were taken from decayed fruit that had been inoculated with pure cultures of *Penicillium italicum* and *P. digitatum* and that showed no outward signs of contamination with other fungi. The fruit was then soaked four minutes in water at 95° and 115° F., allowed to drain and dry on wire racks, and wrapped in individual orange wrappers. Lots of 10 fruits each were placed in net bags for storage at different temperatures.

Separate uninoculated lots of eight fruits each were held at each temperature and weighed at intervals to determine the shrinkage and to serve as checks against the relative humidity readings. Temperatures of other individual fruits were taken by inserting mercury thermometers. A hair hygrometer was used in taking the relative humidity record.

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Except in cases in which decay had started at both ends or the decayed area was so large as to make further keeping impracticable, the fruit was returned to the temperature chambers for further observation. After 12 days at the different temperatures, the lots of fruit were removed from the several chambers and placed in moist jars at about 70° F. for further study of the development of decay.

Table 1 gives the air and fruit temperatures in the various maintained-temperature compartments for the first six days.

TABLE 1.—*Fruit temperatures of California Valencia oranges stored at different air temperatures*

Day of storage	Fruit temperature (° F.) at average [a] air temperature of—						
	50.0° F.	57.7° F.	66.8° F.	74.8° F.	80.6° F.	86.0° F.	88.7° F.
First	80.7	80.7	80.7	80.7	80.7	80.7	80.7
Second	56.5	63.3	70.2	73.9	78.8	85.0	89.1
Third	56.0	63.0	70.2	76.0	80.0	85.5	89.7
Fourth	54.0	66.0	69.2	----	----	86.0	91.8
Fifth	55.0	64.0	70.0	76.0	81.0	86.0	90.0
Sixth	54.0	64.0	69.0	76.0	81.0	85.0	88.5

[a] Average for 12 days.

Table 2 gives average air temperatures, relative humidity, and weight lost by the fruit during the first eight days.

TABLE 2.—*Relative humidity and loss in weight of California Valencia oranges stored at different air temperatures*

Item	Average air temperature [a]						
	50.0° F.	57.7° F.	66.8° F.	74.8° F.	80.6° F.	86.0° F.	88.7° F.
Relative humidity [a]	41.6	53.3	65.4	77.7	85.1	89.7	83.7
Loss in weight per 100 gm. fruit, [b] gm	1.22	1.39	1.55	----	1.6	1.95	4.85

[a] Average for 12 days. [b] Average for 8 days.

GROWTH RATE OF PENICILLIUM ITALICUM AS INFLUENCED BY TEMPERATURE

TABLE 3.—*Growth of Penicillium italicum in orange fruits and in glucose-potato agar at different temperatures*

AVERAGE DIAMETER OF DECAYED SPOTS ON ORANGES

Fruit end and culture	Days	50° F.	57.5° F.	66.8° F.	74.8° F.	80.6° F.	86° F.	88.7° F.
		Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
Stem end	4	1.2	12.5	20.4	28.9	23.7	16.2	10.1
	6	----	28.7	43.1	43.7	41.0	21.1	12.2
	8	24.2	41.4	57.7	59.5	57.8	24.5	12.3
	12	48.0	78.0	----	----	----	36.1	----
Stylar end	4	0.0	1.2	8.5	11.5	8.2	6.5	0.0
	6	----	18.5	26.0	27.0	16.8	9.7	0.0
	8	9.8	26.3	39.7	39.8	24.3	16.8	0.0
	12	27.8	58.2	----	----	----	24.5	----

DIAMETER OF FUNGUS MYCELIAL MAT ON GLUCOSE-POTATO AGAR

Culture	4	11.0	21	24	28	26	12	6
	6	18.5	31	35	37	32	14	7
	8	26.0	40	44	49	36	18	9
	12	35.0	57	67	65	49	20	11

The data in Table 3 and Figure 1 show that *Penicillium italicum* when grown on glucose-potato agar made somewhat the same general response to temperature as when invading the orange fruits. The curves in Figure 1 all have the same optimum point. There is one interesting difference, however, in that the growth on the artificial medium was proportionally faster than on the fruit at the low temperatures, especially in the fruit held four days. From about 75° F. up, the rate on the artificial medium was about the same as at the stem-end half of the fruit. In eight days the decayed areas at the stem-end were almost as large as the mycelial mats in cultures at the lower temperatures and exceeded their growth from about 57° F. up. The average rate of enlargement of decayed spots near the stylar end was very much slower than with those near the stem end at all temperatures during the first eight days. In 12 days the enlargement of decayed spots at the stylar end closely approached that of mycelial mats in cultures. As is seen from Table 1, the fruits at the lower temperatures were slow in cooling down to the temperature of the air in the chamber. This difference in rate of cooling would not seem to account for the lag in enlargement of rot areas as compared with the advance in artificial cultures. In fact a greater lag would be expected if the fruit had cooled as rapidly as the cultures. This difference is probably due to a resistance in the living cells of the fruit to the advance of the invading organism, which resistance is relatively greater at low than at higher temperatures. These data indicate that temperatures between about 65° and 80° are conducive to a rapid rate of decay from *P. italicum* and that at temperatures below and above this range the rate is rapidly retarded. A temperature of 90.5° F. appears to be near the upper limit for growth. A temperature of 50° F. appears to be considerably above the lower limit, but greatly retards the rate of development of decay.

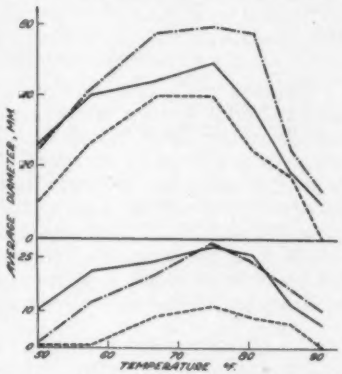

FIG. 1—Growth of *Penicillium italicum* in relation to temperature and to location of inoculation. (Lower graphs, 4-day growth; upper graphs, 8-day growth)
At stylar half of fruit - - - - - - -
At stem-end half of fruit — — — — —
On glucose-potato agar ———————

A marked difference in rate of decay is shown between the stem and the stylar half of the fruit. The shape of the graphs of the enlargements, however, is very similar, indicating the same optimum point near 75° F. with a retardation of rate at lower and higher temperatures in much the same way in both cases.

GROWTH RATE OF PENICILLIUM DIGITATUM AS INFLUENCED BY TEMPERATURE

TABLE 4.—*Growth of Penicillium digitatum in glucose-potato agar at different temperatures*

Days	Diameter of mycelial disks at—						
	50° F.	57.5° F.	66.8° F.	74.8° F.	80.6° F.	86° F.	90.5° F.
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
Second	0	6.7	13.0	14.5	15	5.0	0
Fourth	6	20.0	35.0	38.0	30	5.0	0
Sixth	13	33.0	53.5	56.0	37	6.5	0
Eighth	22	53.0	75.0	76.0	51	7.5	0

The data in Table 4 and Figure 2 indicate that *Penicillium digitatum* also has an optimum temperature somewhere near 75° F., and possibly, as judged from the shape of the graphs, the optimum is a little lower than that of *P. italicum*. The retardation of growth in passing from the optimum to lower and higher temperatures appears to be relatively more rapid than with *P. italicum*. The upper limit appears to be below 90.5° F., since no growth took place at that temperature. At 50° F. a marked retardation is evident. The rate of spread of this fungus was so rapid that measurements on the fruit corresponding to those for *P. italicum* were not feasible.

DECAY OF FRUIT BY PENICILLIUM ITALICUM

TABLE 5.—*Penicillium italicum decay on inoculated California Valencia oranges stored at different temperatures, showing relative susceptibility of stem and stylar end of fruit*

Temperature of air (°F.)	Number of fruits	Percentage of injuries showing decay after—							
		4 days		8 days		12 days		28 days	
		Stem end	Stylar end	Stem end	Stylar end	Stem end	Stylar end	Stem end	Stylar end
50	10	30	0	100	60	100	90	.....	.....
	10	0	0	70	20	100	100	.....	.....
	10	0	0	80	10	100	90	.....	.....
Average	.....	10	0	83.3	30	100	93.3	.....	.....
57.5	10	90	10	100	90	100	100	.....	.....
	10	90	60	100	100	.....	.....	.....	.....
	10	60	20	100	100	.....	.....	.....	.....
Average	.....	80	30	100	96.7	.....	.....	.....	.....
66.8	10	100	60	100	100	.....	.....	.....	.....
	10	100	70	100	100	.....	.....	.....	.....
	10	60	30	90	90	.....	.....	.....	.....
74.8	10	100	60	100	100	.....	.....	.....	.....
80.6	10	100	60	100	70	.....	.....	.....	.....
Average	.....	92	56	98	92	.....	.....	.....	.....
86	10	100	50	100	60	100	70	.....	.....
	10	90	0	100	0	100	0	.....	.....
	10	90	30	100	30	100	30	.....	.....
Average	.....	93.3	26.7	100	30	100	33.3	.....	.....
88.7	10	0	0	0	0	0	0	40	50
	10	40	10	50	50	50	50	60	70
	10	60	10	90	40	90	40	90	40
Average	.....	33.3	6.7	46.7	30	46.7	30	63.3	53.3

Differences in temperature produced noticeable differences in the quantity and location of decay and in the time required for the injuries to soften. Table 5 gives the percentage of injuries showing decay on Valencia orange fruits inoculated with *Penicillium italicum* at the stem and stylar end. Figure 3 contains a graphical presentation of the average percentages. Practically all the fruit held at temperatures ranging from 57.5° to 86° F. decayed in 4 or 8 days, but when held at 50° total decay required from 8 to 12 days. At 88.7° the amount of decay was below 50 per cent at the end of 12 days, and increased to only a little over 60 per cent during 16 additional days in 70° moist jars. This fact suggests a possible killing of some spores by the high temperature. In fact, in one experiment 90 per cent of the fruit started to soften at the injuries during the first four days, but all of these spots became dry and showed no advance or spore formation during the rest of the high-temperature storage period, and only about 50 per cent of the inoculations developed decay after the fruit was removed to 70° moist jars.

The difference in amount of decay present in injuries at the stem and stylar ends is found mainly in the period of maximum production of decay. At temperatures from 57.5° to 80.6° F. practically all fruits were decayed at both ends in 8 days, although the maximum production of decay at the stem end occurred during the first 4 days while that at the stylar end occurred during the second 4 days. At 50° practically all the fruits were decayed at both ends in 12 days, although the maximum production of decay occurred at the stem end during the second 4-day period while that at the stylar end occurred during the third period. The fruit held at 86° showed decidedly less decay at the stylar end than at the stem end and the difference was consistent throughout three experiments. At 88.7° the percentage of decay was slightly less at the stylar end than at the stem end, and the maximum development of decay at the stylar end came during the 4-day period following that at which it occurred at the stem end.

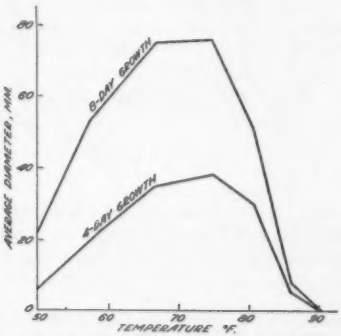

FIG. 2.—Growth of *Penicillium digitatum* on glucose-potato agar in relation to temperature

DECAY OF FRUIT BY PENICILLIUM DIGITATUM

Table 6 gives the percentage of injuries showing decay due to *Penicillium digitatum* at stem and stylar end of Valencia oranges. These averages are graphically presented in Figure 3. The decay of orange fruits by *P. digitatum* apparently is affected more by differences in temperature than is that by *P. italicum*. There appears to be relatively less decay by *P. digitatum* at temperatures above 80.6° F. and below 66.8°. This difference in general parallels the relative difference in rate of growth of the two organisms at the various temperatures. At temperatures of 66.8° to 80.6° practically

all the fruit decayed from *P. digitatum* in four days, while at 50° to 57.5° and at 86° only from 70 to 80 per cent of the fruit decayed during the first four days and very little additional decay developed

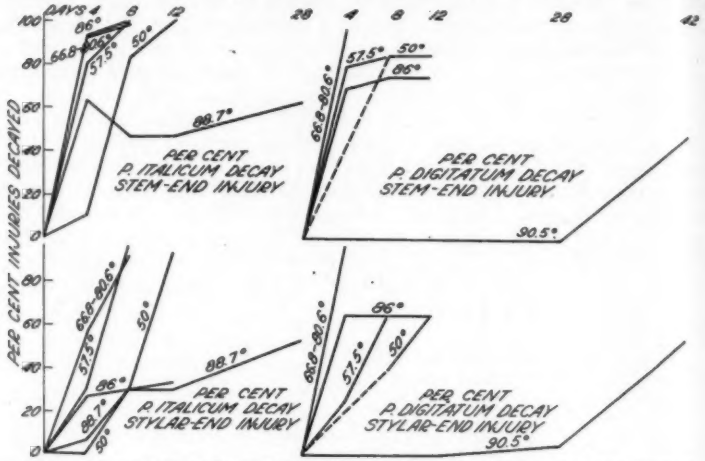
PER CENT INJURIES DECAYED
DAYS 4 8 12 28 4 8 12 28 42
86°
66.8-80.6°
57.5°
50°
88.7°
PER CENT P. ITALICUM DECAY STEM-END INJURY
PER CENT P. DIGITATUM DECAY STEM-END INJURY
90.5°
PER CENT P. ITALICUM DECAY STYLAR-END INJURY
PER CENT P. DIGITATUM DECAY STYLAR-END INJURY

FIG. 3.—Average decay of California Valencia oranges at stem and stylar end during storage at 50° to 90.5° F.

later. No fruit decayed at 90.5° and only about half the fruits developed decay during 30 additional days at 70°. Thus with *P. digitatum* greater inhibition is shown at 90.5° than occurred with *P. italicum* at 88.7°.

TABLE 6.—*Penicillium digitatum decay on inoculated California Valencia oranges stored at different temperatures, showing relative susceptibility of stem and stylar end of fruit*

Temperature of air (°F.)	Number of fruits	Percentage of injuries showing decay after—									
		4 days		8 days		12 days		28 days		42 days	
		Stem end	Stylar end	Stem end	Stylar end	Stem end	Stylar end	Stem end	Stylar end	Stem end	Stylar end
50	10	.....	.....	100	50	100	100	.....	.....	.....	.....
	10	.....	.....	70	30	70	30	.....	.....	.....	.....
Average	.....	.....	.....	85	40	85	65	.....	.....	.....	.....
57.5	10	90	20	100	100	.....	.....	.....	.....	.....	.....
	10	70	30	70	30	.....	.....	.....	.....	.....	.....
Average	.....	80	25	85	65	.....	.....	.....	.....	.....	.....
66.8	10	100	100	.....	.....	.....	.....	.....	.....	.....	.....
	10	100	100	.....	.....	.....	.....	.....	.....	.....	.....
74.8	10	100	100	.....	.....	.....	.....	.....	.....	.....	.....
	10	100	90	.....	.....	.....	.....	.....	.....	.....	.....
80.6	10	100	90	.....	.....	.....	.....	.....	.....	.....	.....
	10	80	100	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	96.7	96.7	.....	.....	.....	.....	.....	.....	.....	.....
86	10	80	60	80	60	80	60	.....	.....	.....	.....
	10	60	70	70	70	70	70	.....	.....	.....	.....
Average	.....	70	65	75	65	75	65	.....	.....	.....	.....
90.5	10	0	0	0	0	0	0	0	10	30	40
	10	0	0	0	0	0	0	0	0	70	70
Average	.....	0	0	0	0	0	0	0	5	50	55

At temperatures ranging from 66.8° to 86° F. decay at the stylar end was more rapid with *Penicillium digitatum* than with *P. italicum*. As much decay was produced at stylar-end injuries by *P. digitatum* in the same time at temperatures of 66.8° to 86° as was produced at stem-end injuries. At temperatures of 50° to 57.5° the maximum production of decay at the stylar-end injuries occurred one 4-day period later than maximum decay at the stem-end injuries.

SUMMARY

Penicillium italicum and *P. digitatum*, the two fungi most commonly producing soft rot in citrus fruits, are in general affected similarly by different temperatures.

On culture media as well as on orange fruits both appear to have nearly the same optimum temperature for development. The differences, however, between the rate of growth at the optimum as compared with the rates at higher and lower temperatures are greater with *Penicillium digitatum* than with *P. italicum*.

In most cases the rate of development of decay was much more rapid at the stem end than at the stylar end of the orange fruits. The percentage of decay in most cases was also greater from injuries near the stem end than from equal injuries near the stylar end.

In lots of fruit inoculated with *Penicillium italicum* and held, respectively, at 66.8°, 74.8°, and 80.6° F., nearly all fruits showed decay in 4 days, while lots held at 86° and 57.5° showed similar decay in 8 days, and lots held at 50° showed similar decay in 12 days.

In lots inoculated with *Penicillium digitatum* much the same general relation of temperature to time and percentage of decay was noted except that apparently the temperatures above and below the optimum had a greater relative inhibiting effect than in the lots inoculated with *P. italicum*.

THE RESISTANCE OF CERTAIN VARIETIES OF WINTER WHEAT TO ARTIFICIALLY PRODUCED LOW TEMPERATURES[1]

By DONALD D. HILL, *Instructor in Agronomy*, and S. C. SALMON, *Professor of Farm Crops, Kansas State Agricultural College*

INTRODUCTION

As is well known, injury to wheat by winterkilling results in severe losses in the winter-wheat-producing regions of the United States. The exact loss is difficult to estimate because it is so easily confused with injury from other causes such as drought, soil blowing, Hessian fly, etc. According to estimates made by the United States Department of Agriculture,[2] the average loss from frosts or freezing for the 14-year period 1909 to 1922 was 3.5 per cent. According to the same authority, the average acreage abandoned for the 23-year period from 1901 to 1923 was approximately 10 per cent. Much, but not all, of this abandonment was due to winterkilling.

The problem of winter hardiness is somewhat more important than these estimates suggest, since there is involved not only the actual loss but also the potential gain that might be derived from growing winter wheat in what is now regarded as spring-wheat territory were it possible to secure varieties that would survive the winters.

Varieties are known which are considerably more hardy than those generally used in commercial production. They have certain defects, however, such as late maturity, inferior quality, and low yields, which more than offset their greater hardiness for most conditions.

These facts suggest that breeding wheat for cold resistance may properly be considered a project of major importance and worthy of serious attention. In such a project the determination of the relative hardiness of varieties now in existence, as well as of any new ones that may be produced, is of first importance. This, however, is a difficult matter because of the variations in seasons as well as other sources of error. Some winters are so mild that the least hardy varieties survive and others are so severe that all are killed. In either case, nothing is learned as to their relative winter hardiness. At the Kansas station, for example, nine successive winters have been so mild that no information on relative winter hardiness has been obtained. It is known from previous experience, though, that a variety to be generally satisfactory in Kansas must possess a reasonably high degree of winter hardiness. The importance of the problem is further emphasized by the fact that at the present time approximately 4,000,000 acres of Blackhull, a variety of questionable winter hardiness, is being grown in this State. It is believed that the success of this variety has been partially due to the mild winters prevailing in Kansas during the past few years. These facts suggest the urgent need of some method of determining the relative winter hardiness more quickly and more certainly than can be done by field trials.

[1] Received for publication Oct. 11, 1927; issued December, 1927. Contribution No. 172, Department of Agronomy, Kansas State Agricultural College.

[2] UNITED STATES DEPARTMENT OF AGRICUTURE. AGRICULTURAL STATISTICS. BREAD GRAINS. U. S. Dept. Agr. Yearbook 1923: 601–661. 1924.

Many attempts have been made to correlate cold resistance with some easily observed morphological or physiological character. The relation of winter hardiness to the size of the cells, the habit of growth, osmotic pressure of the cell sap, the water and sugar content of the tissue, the hydrophilic colloids of the protoplasm, and other characters have been studied. No results of great value have been secured, so far as finding a practical means of detecting winter-hardy varieties or strains is concerned.

EXPERIMENTAL DATA

During the past year an attempt was made at the Kansas Agricultural Experiment Station to determine the feasibility of artificially freezing plants as a means of determining their relative hardiness. The general plan was to grow varieties of known hardiness and subject them to low temperatures under controlled conditions. The low temperatures were secured with a carbon dioxide direct-expansion refrigeration plant, in which temperatures were controlled automatically. In a preliminary trial 25 varieties were studied, but it seemed desirable in later experiments to limit the study to 10, so selected as to secure a rather wide range in winter hardiness. The plants were grown in the greenhouse and subjected to various degrees of cold for different periods of time with and without previous hardening and with various degrees of soil moisture. Part of the plants were grown in greenhouse flats and part in 4-inch clay pots. After freezing, the plants were thawed gradually and then placed in the greenhouse with suitable temperatures for growth. The apparent injury to the plants was recorded from 5 to 7 days after freezing and the final survival 10 to 14 days later. About 100 trials of 100 plants each were made, of which about 75 per cent were hardened before freezing. Table 1 gives the average survival of about 50 trials in which the plants were hardened for various periods of time previous to freezing. The relative average survival of nine of these varieties grown in the uniform winter-hardiness nurseries of the United States Department of Agriculture for the two years 1925–26 and 1926–27 is also given for comparison.[3]

TABLE 1.—*Average survival of wheat varieties expressed as a percentage of Kharkof*

Variety	Artificial refrigeration		Uniform winter-hardiness nursery: Average 1925–26 and 1926–27	
	Survival	Rank	Survival	Rank
	Per cent		*Per cent*	
Minhardi	96	4	123.9	1
Buffum	104	1	122.5	2
Minturki	88	5	116.5	3
Kharkof	100	2	100.0	4
Kanred	100	2	95.9	5
Harvest Queen	80	6	87.7	6
Tenmarq	73	7	85.6	7
Blackhull	57	8	77.2	8
Fulcaster	54	9	71.5	9
Nebraska No. 28	47	10		

[3] Data kindly furnished by J. Allen Clark, J. H. Martin, and J. H. Parker.

Nebraska No. 28 was not included in the uniform winter-hardiness nurseries in these two seasons, but from previous work [4] and from data secured at Manhattan it is known that this variety is relatively nonwinter hardy as measured by field tests. It may be seen that excepting the two very hardy varieties, Minhardi and Minturki, the results for the two methods of testing are in very close agreement. In general the failure to agree in all respects is not surprising, considering (1) the variation in the comparative hardiness from year to year and from place to place in the field trials; (2) the fact that in field trials, factors other than degree of cold undoubtedly played a part; and (3) that in the artificial refrigeration trials, the plants were only partially hardened. The periods of hardening were in no case longer than 19 days, and often only 3 or 4 days with temperatures seldom or never below 20° F.

The usefulness of artificial refrigeration was also shown in trials comparing Kanred and Blackhull. The popularity and large acreage of the latter variety in the southern Great Plains suggested extensive trials with it in comparison with Kanred. Plants of these two varieties were dug up from field plots in January, placed in small greenhouse flats, one-half of each flat being Kanred and the other Blackhull, and subjected to a minimum temperature of approximately −10° F. for various periods of time. Table 2 gives the detailed results.

TABLE 2.—*Relative survival of Kanred and Blackhull wheat when subjected to low temperatures*

Lot No.	Variety	Hours frozen	Apparent injury at end of—		Survival
			7 days	15 days	
			Per cent	*Per cent*	*Per cent*
1	Kanred	6	10	0	100
	Blackhull	6	10	0	100
2	Kanred	12	20	30	100
	Blackhull	12	50	80	40
3	Kanred	15	15	20	100
	Blackhull	15	50	98	2
4	Kanred	18	30	30	100
	Blackhull	18	50	100	0
5	Kanred	21	50	70	60
	Blackhull	21	80	100	0
6	Kanred	24	60	90	10
	Blackhull	24	85	100	0

It will be observed that the results are in close agreement with what is known of the relative survival of these varieties as determined in the uniform winter-hardiness nurseries. Altogether the results of the artificial refrigeration trials indicate that the method is a very promising one for studying the relative hardiness of different varieties.

A very interesting and equally important factor in the study of winter hardiness is the degree of hardening to which the plants are subjected before they are frozen. This is illustrated in Table 3, in which are given the results of 17 trials with greenhouse-grown plants subjected to freezing temperatures without previous hardening.

[4] CLARK, J. A., MARTIN, J. H., and PARKER, J. H. COMPARATIVE HARDINESS OF WINTER-WHEAT VARIETIES. U. S. Dept. Agr. Circ. 378, 20 p., illus. 1926.

TABLE 3.—*Average survival in 17 freezing trials with unhardened plants, expressed as a percentage of Kharkof*

Variety	Percentage survival	Rank
Minhardi	66	6
Buffum	74	3
Minturki	69	5
Kanred	100	1
Kharkof	100	1
Harvest Queen	71	4
Tenmarq	46	9
Blackhull	54	7
Fulcaster	47	8
Nebraska No. 28	28	10

The average survival of the three varieties Minhardi, Buffum, and Minturki, known to be hardiest under field conditions, was but little better than that of such varieties as Blackhull, Harvest Queen, and Fulcaster, known to be relatively low in the winter-hardiness scale. This suggests not only that hardening is very important in determining survival but also that different varieties react very differently in this respect. It is probable that had the experimental plants been more thoroughly hardened before freezing, the results presented in Table 1 would have been much more favorable for those known to be winter-hardy under field conditions. It seems quite likely that hardiness is due to the ability of certain varieties to build up, in some way, a high degree of protection from cold, whereas other varieties lack this ability. Observations made on individual lots support this idea in that hardy varieties appeared to the best advantage in those cases where hardening was more nearly complete.

The effect of differences in hardening is also indicated by data obtained from freezing individual lots of Kanred which had either not been hardened or had been subjected to two conditions of hardening, namely, mild or greenhouse hardening and field hardening. The data presented in Table 4 show how little of the maximum hardiness had been built up by the greenhouse hardening. The difference between the mild-hardened and the field-hardened plants is especially marked. In view of the behavior of the hardier varieties in these trials it is not unreasonable to suppose that the difference might be still greater with other varieties.

TABLE 4.—*Freezing trials with Kanred unhardened and hardened for different periods*

Treatment	Mean temperature	Period of freezing	Survival
	°F.	*Hours*	*Per cent*
Unhardened	0	2.5	70
Mild hardened	0	5.0	70
Field hardened	−10	18.0	100
Do	−10	21.0	60

In a general way, it appears that the 10 varieties may be placed, tentatively at least, in three groups. (1) Varieties which are relatively hardy when thoroughly hardened before freezing. This group

includes Minhardi, Minturki, and Buffum. (2) Varieties which are relatively hardy when hardened before freezing but which also carry a relatively high degree of hardiness in the unhardened condition. In this class are Kanred, Kharkof, and possibly Harvest Queen. (3) Varieties which are relatively nonhardy regardless of whether they are hardened prior to freezing. Such varieties are Blackhull, Nebraska No. 28, Fulcaster, and probably Tenmarq.

The relation of the moisture content of the soil to resistance to freezing injury was also investigated in a number of trials. In all cases, it was found that plants growing in a dry soil were injured much more severely than similar plants in a wet soil. Plants dried to the point of wilting and watered just before freezing survived as well as those which had been abundantly supplied with water at all times. This is easily explained by the high specific heat of water which in a wet soil prevents a rapid change in temperature, the net result being that those plants in dry soil are exposed to a lower temperature than those in wet soil. These results confirm the general observation that winterkilling is frequently more severe in dry soils than in those well supplied with moisture, excepting, of course, those cases where winterkilling is a result of heaving or smothering rather than of low temperature. These relations would seem to be particularly important in determining artificially the relative winter hardiness of varieties. It is rather difficult to maintain a uniform moisture content under greenhouse conditions, and if this matter is not carefully considered, differential results in freezing may be due as much to differences in moisture content of the soil as to differences in varieti es

RELATION BETWEEN WATER AND POTASH IN PLANT PRODUCTION [1]

By FRED W. MORSE [2]

Massachusetts Agricultural Experiment Station

INTRODUCTION

The beneficial effects of potash fertilizers in dry seasons are mentioned by Hall in his description of the Rothamsted experiments.[3] The relation between potash fertilizers and the economical use of water by crops has also been discussed by Von Seelhorst.[4] Hall considers that plentiful potash prolongs the growth of the plant and offsets the ripening action of the phosphoric acid, which in the absence of potash acts prematurely and the action is intensified by the heat and dryness. The opinion of Von Seelhorst is that the potash effect is in accord with the law of the minimum, and he shows that if nitrogen or phosphorus is the scarce element, its addition will likewise produce a more efficient use of water by the plant.

As part of a study of the availability of the potash naturally present in some typical soils, observations were made on the relations between the water supply and potash supply by means of pot experiments with soy beans and Japanese millet. These two crops are distinctly different types and are admirably suited to growth in pots during the summer.

Wagner pots [5] 25 cm. in diameter and 33 cm. in depth were used in these experiments.

EXPERIMENTS OF THE FIRST SEASON

For the first season the soil was taken from a field of the experiment station called "field G," which for 30 years had been used for comparative studies of potash fertilizers. The soil was a fine sandy loam belonging to the Merrimac series and was a bit heavy for pot experiments.

One lot of soil was taken from the surface of two plots that had been without potash fertilizers for over 30 years, but had been liberally supplied with nitrogen and phosphates. A second lot of soil was mixed from the surface of two plots which had received potash fertilizers annually in addition to the other chemicals—in the one case, muriate of potash; in the other, sulphate. From these two

[1] Received for publication Oct. 4, 1927; issued December, 1927. Published by permission of the director of the Massachusetts Agricultural Experiment Station.

[2] Credit is due R. L. Coffin for the execution of all details of the pot experiments and to H. D. Haskins and L. S. Walker for the chemical analyses of the crops.

[3] HALL, A. D. THE BOOK OF THE ROTHAMSTED EXPERIMENTS. Ed. 2, rev. by E. J. Russell. p. 59, 87. London, J. Murray. 1917.

[4] SEELHORST, C. VON. WIE WIRKT EINE KALIDÜNGUNG AUF DEN WASSERVERBRAUCH DER PFLANZEN UND AUF DEN WASSERGEHALT DER ERDE. Jour. Landw. 63: [345]-356. 1915.

[5] LINDSEY, J. B. WAGNER METHOD OF POT CULTURE. U. S. Dept. Agr., Expt. Sta. Rec. 7: 77-87, illus. 1895.

lots of soil there were prepared four different series of pots for comparison, viz: Series A, soil from plots without potash; series B, with potash added to the soil of series A; series C, soil from plots with potash residues; and series D, with potash added to the soil of series C.

By this arrangement, a soil considerably exhausted of its natural potash was compared with a soil containing the residual potash of 30 years' continuous applications of that substance in the fertilizers, and with soluble salts of potash added to both.

Each lot of soil was thoroughly mixed; the amount for each pot was weighed and the chemicals allotted were mixed with it before the pot was filled.

The amounts of fertilizer chemicals used in every pot consisted of 1.5 gm. ammonium nitrate and 3 gm. double superphosphate. The pots which received potash were each allotted 1 gm. of potassium sulphate. These quantities were calculated to be proportional to the amounts per acre used annually on the plots.

Three different quantities of water were used, as follows: A maximum supply calculated to be 50 per cent of the water-holding capacity of the soil, a medium supply of 37.5 per cent, and a minimum supply of 25 per cent.

After the addition of its allotment of water, the gross weight of the pot was thereafter used as the basis for maintaining the supply of soil-moisture approximately uniform from day to day.

Seeds were planted in all the pots on June 7. Germination was completed in a week, and as growth progressed surplus plants were removed until there were left five equidistant plants in each pot.

Early in their growth, both crops began to show the effects of the variations in potash and water supplied them. After about three weeks, the millet plants with the maximum supply of water began to lag behind those with the medium supply, continued to be inferior in size throughout the entire period, and barely surpassed the millet with minimum water supply. Heads appeared first on the millet with the maximum supply of water, while the plants with the minimum supply were the last to show them. Full height of the millet was reached in all pots by August 7, and the plants were harvested on August 30.

The soy beans continued throughout the season to show the effects of the variations in water supply upon the size of the plants. The soy-bean plants that grew in the soils which contained potash ripened normally and were harvested on September 26. The plants in the soil which had received no potash for many years grew abnormally. They remained green and the pods did not fill well. They were not harvested until October 6, when some leaves were yet green and the pods were badly rusted.

When harvested the crop of each pot was put in a cheesecloth sack and hung in the glasshouse, where the bags remained undisturbed, exposed to sun and air, until the chemical analyses could be made.

The air-dry crops were weighed and analyzed. The weights of the crops from duplicate pots agreed well in all the pairs and calculations were based on the combined crops of each pair of pots. Seed and straw were weighed separately with each crop. The millet seed and

straw were remixed and analyzed only as crops. The soy beans from one of each pair of pots of series A and B were analyzed as separated into seeds, pods, and straw because of the marked difference in development between the plants with potash and those without it. The plants from the duplicate pot were remixed and analyzed as a crop.

It was found by several trials that the moisture content of the different crops was quite uniform; consequently, to save labor, subsequent analyses and calculations were all based on the air-dry state.

TABLE 1.—*Weights of air-dry crops and percentages of nitrogen and potash contained in them; first-season experiments*

Crop and series	Water supply	Weight of crop			Nitrogen	Potash
		Seed	Straw	Total		
Millet:		*Grams*	*Grams*	*Grams*	*Per cent*	*Per cent*
Series A, soil from plots without potash	Maximum	46	40	86	1.21	0.90
	Medium	55	51	106	.94	.72
	Minimum	52	50	102	1.08	.56
Series B, potash added to series A	Maximum	40	42	82	1.05	1.39
	Medium	58	64	122	.94	.94
	Minimum	44	60	104	.97	.99
Series C, soil from plots with potash residue	Maximum	44	47	91	.93	1.65
	Medium	66	80	146	.84	1.39
	Minimum	53	76	129	.81	1.51
Series D, potash added to series C	Maximum	46	50	96	.82	1.82
	Medium	61	76	137	.87	1.72
	Minimum	55	74	129	.83	1.66
Soy beans:						
Series A, soil from plots without potash	Maximum	48	180	228	2.60	.41
	Medium	49	159	208	2.66	.47
	Minimum	37	107	144	2.90	.55
Series B, potash added to series A	Maximum	96	191	287	2.62	.61
	Medium	82	176	258	2.47	.66
	Minimum	55	108	163	2.65	.72
Series C, soil from plots with potash residue	Maximum	113	222	335	2.09	.91
	Medium	99	195	294	2.35	1.08
	Minimum	60	115	175	2.56	1.10
Series D, potash added to series C	Maximum	110	224	334	2.44	1.14
	Medium	103	201	304	2.34	1.13
	Minimum	60	114	174	2.36	1.28

The outstanding result with millet was the failure of the maximum supply of water to produce the largest plants, although the concentration of potash in them was highest in each series. (Table 1.) The millet in series A, on soil exhausted of potash, yielded by weight more seed than straw. Increased supplies of potash increased the straw proportionally more than the seed.

Soy beans showed a different relation to both potash and water. The maximum supply of water in all series produced the maximum crop, both straw and seed being increased proportionally. The concentration of potash was highest in the crops with least water. Addition of potash to the soil exhausted of potash gave a marked increase in seed, more proportionally than of straw, especially with plenty of water.

TABLE 2.—*Weight of seeds, pods, and straw of soy beans, together with percentages of nitrogen and potash in each, showing the effect of varied supplies of water and of potash*

Item	Weight of crop			Nitrogen in—			Potash in—		
	Seeds	Pods	Straw	Seeds	Pods	Straw	Seeds	Pods	Straw
Series A, without potash:	*Grams*	*Grams*	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Maximum water	48	44	136	6.25	1.68	1.61	1.17	0.15	0.23
Medium water	49	39	120	6.47	1.60	1.43	1.25	.15	.25
Minimum water	37	26	81	6.88	1.57	1.53	1.26	.20	.35
Series B, with potash:									
Maximum water	96	57	134	6.25	.68	.87	1.49	.12	.21
Medium water	82	50	126	6.16	.63	.81	1.65	.37	.15
Minimum water	55	29	79	6.46	.51	.79	1.80	.10	.20

In Table 2 it may be noted that with an abundance of water the addition of potash doubled the weight of seed, increased the weight of pods somewhat, and did not affect the straw, which consisted of the stems and leaves. This in general is the effect with the two series, although as the water supply diminished the addition of potash was less efficient.

The composition of the different parts of the plants is striking in its variations. Seeds are fairly uniform in percentages of nitrogen. The addition of potash produces a somewhat higher percentage of that constituent in series B. Without potash, the pods and straw of series A contain approximately twice as much nitrogen as their corresponding parts in series B. Potash is fairly uniform throughout both series.

The evidence indicates that potash was needed to aid the translocation of nitrogen from stems, leaves, and pods into the seeds, and by helping in this transfer, greatly increased the weight of seeds.

EXPERIMENTS OF THE SECOND SEASON

The pot experiments were conducted a second season with some modifications. In addition to the soils from field G, two lots of soil were procured from a field that had been especially responsive to potash in the fertilizer, and has been described in the reports of the experiment station as the "north soil test."[6] One lot of soil was taken from the plot which had received no potash for 30 years and the other lot was from the plot which had received the complete fertilizer, including muriate of potash. Both plots had always been dressed with moderate quantities of nitrate of soda and acid phosphate. By adding potash to each of these soils, four series of soils were prepared: Series A from the plot without potash; series B with potash added to A; series C from the plot with potash; and series D with potash added to C.

Larger amounts of the respective fertilizer chemicals were used this year in all the pots because the quantities employed in the previous season, although proportional to the field applications, were much smaller than experience had shown to be optimum for restricted volumes of soil in pots. Each pot of millet received 6 gm. ammonium nitrate, 7 gm. double superphosphate, and 6 gm. limestone, while

[6] HASKELL, S. B. A THIRTY-YEAR FERTILIZER TEST. Mass. Agr. Expt. Sta. Bul. 212, p. [127]–158, illus. 9122.

the pots of soy beans were given 3 gm. ammonium nitrate, 7 gm. double superphosphate, and 3 gm. limestone. The potash was supplied to the designated pots in 8 gm. sulphate of potash.

The maximum supply of water for the millet was 50 per cent of the water-holding capacity of the soil, while for soy beans it was raised to 60 per cent. The medium supply was 35 per cent for both crops. Millet received as a minimum supply only 20 per cent of the water-holding capacity, but soy beans received 25 per cent, as in the first season. The water-holding capacity of the soils ranged between 52 and 54 per cent, so that they were practically alike in that property.

TABLE 3.—*Weights of air-dry crops and percentages of nitrogen and potash contained in them; second season experiments*

Crop and series	Water supply	Weight of crop			Nitrogen	Potash
		Seed	Straw	Total		
Millet:		*Grams*	*Grams*	*Grams*	*Per cent*	*Per cent*
Series A, soil from plots without potash	Maximum	75	64	139	0.88	0.77
	Medium	68	63	131	.91	.62
	Minimum	66	56	122	1.60	.49
Series B, potash added to series A	Maximum	91	103	194	.85	1.73
	Medium	90	107	197	.73	1.89
	Minimum	92	112	204	1.24	2.10
Series C, soil from plots with potash residue	Maximum	95	97	192	.90	1.60
	Medium	87	97	184	.80	1.59
	Minimum	96	95	191	1.37	1.52
Series D, potash added to series C	Maximum	103	111	214	.86	1.87
	Medium	88	111	199	.77	2.12
	Minimum	90	107	197	1.38	2.38
Soy beans:						
Series A, soil from plots without potash	Maximum	45	158	203	3.05	.72
	Medium	41	132	173	2.65	.59
	Minimum	35	97	132	2.88	.55
Series B, potash added to series A	Maximum	154	257	411	3.10	1.63
	Medium	103	191	294	2.78	1.80
	Minimum	74	132	206	2.90	1.82
Series C, soil from plots with potash residue	Maximum	153	240	393	3.16	1.23
	Medium	101	181	282	2.93	1.43
	Minimum	67	127	194	2.84	1.28
Series D, potash added to series C	Maximum	152	238	390	2.66	2.20
	Medium	92	178	270	2.72	2.07
	Minimum	75	129	204	2.76	1.99
Millet—North soil test series:						
Series A, soil from plot without potash	Maximum	86	72	158	1.03	.30
	Medium	55	52	107	.95	.36
	Minimum	59	54	113	1.53	.26
Series B, potash added to series A	Maximum	133	134	267	.90	1.87
	Medium	82	93	175	.87	2.10
	Minimum	123	116	239	1.19	2.00
Series C, soil from plots with potash residue	Maximum	92	97	189	.98	.86
	Medium	105	105	210	1.06	.73
	Minimum	106	100	206	1.16	.65
Series D, potash added to series C	Maximum	101	111	212	.99	2.23
	Medium	83	96	179	.87	2.38
	Minimum	124	117	241	1.04	2.32

Pots were filled and handled as in the first season. Seeds were sown June 20. Germination was prompt and the plants were soon thinned to five in each pot. Growth without potash was again inferior but was proportional to the water supply in the soy beans, while millet showed little difference between maximum and medium supplies.

The millet was harvested on September 12. Plants with the minimum water supply were least matured and numerous heads were imperfect. The pots which received the medium supply of water in series B and D of the north soil test did not yield good parallel weights.

The soy beans grown in series A with little available potash again failed to mature with the rest of the crop. The plants on this soil that received the most water showed many leaves with edges yellow and somewhat curled. The soy beans in all the pots except those just mentioned were harvested 115 days after seeding, but the plants in series A were grown 120 days before cutting, which was performed on October 18.

The crops were dried, weighed, and analyzed as described in the first experiment. The results are shown in Table 3.

The soils of series A that had been without potash fertilizers for years showed a low availabliity of their natural potash. The percentages of potash in the crops were very low. The soils of series C with their residual potash gave yields about equal to those produced by additions of potash, but with somewhat lower percentages of potash in the crops than were present in those from the addition of potash.

With plenty of available potash, the variations in water supply produced no proportional variations in yields of crops of millet, but soy beans were as much influenced by water supply as by potash. For illustration, in series B with added potash and maximum water supply, the yield of soy beans was double that in series A without the potash; also in series B, the yield with the maximum water supply was double that with the minimum supply. Approximately the same differences in results are to be observed between maximum and minimum water supplies in series C and D.

The effect of potash on seed production in the soy beans was even more striking than in the first season. With an abundance of water the weights of seed with potash were triple the yield without potash.

The effect of potash on the seed production of millet was a repetition of the first season also. Without potash in series A, the yield of seed exceeded the weight of straw. With potash in series B, the straw exceeded the seed. Seed was increased by potash from 20 to 40 per cent, while straw was increased from 60 to 100 per cent.

When the crops from the three series with available potash are compared with those from series A, it may be noted that the crops of millet from the available potash with the least water exceed in weights of seed and of straw the crop in series A with the most water.

Soy beans when similarly compared show a marked increase in seed from the available potash with least water but no increase in the straw. Of these pot experiments it can be said that the presence of potash increased the efficiency of the limited supply of water in developing the plants.

In Table 4 are given the weights of potash actually recovered from the soil in each of the crops. In the first column—series A, without potash for years—it is clearly evident that the availability of the potash is dependent on the water supply, as the amount recovered with the minimum supply of water is much less than that recovered with the maximum supply; while additions of potash to the soil give large increases in the potash found in the crops, undoubtedly due to the greater concentration of the soil solution.

Both variables, potash and water, affected the two crops in these experiments in quite different ways. These different effects are shown most clearly in the second season when nitrogen and phosphorus were in ample supply.

TABLE 4.—*Weights of potash recovered in crops grown with varied supplies of potash and of water*

Crop and season	Water supply	Potash recovered from—			
		Series A (without potash)	Series B (with potash added to A)	Series C (with potash residue)	Series D (with potash added to C)
		Grams	*Grams*	*Grams*	*Grams*
Millet, first season	Maximum	0.77	1.14	1.51	1.74
	Medium	.76	1.15	2.03	2.35
	Minimum	.57	1.03	1.93	2.14
Soy beans, first season	Maximum	.93	1.75	3.05	3.81
	Medium	.98	1.70	3.17	3.44
	Minimum	.79	1.17	1.93	2.23
Millet, second season	Maximum	1.07	3.35	3.07	4.02
	Medium	.81	3.72	2.92	4.22
	Minimum	.60	4.28	2.90	4.69
Soy beans, second season	Maximum	1.45	4.70	4.83	8.58
	Medium	1.02	5.29	4.03	5.60
	Minimum	.72	3.75	2.49	4.10
Millet, second season (north soil test soil)	Maximum	.47	4.99	1.62	4.72
	Medium	.38	----------	1.53	----------
	Minimum	.29	4.80	1.34	5.59

In Table 3 a comparison of the yields of millet in series A with those in series B shows the gains resulting from the addition of potash to be greatest with the minimum supply of water, while a similar study of the soy-bean crops shows them to have been most responsive to potash with the maximum supply of water.

These differences in their relations to potash and water are corroborated in Table 4 by the weights of potash recovered in the crops in the second season.

The soy beans recovered the most potash when supplied with the maximum quantity of water. Millet recovered the most potash from series B and series D when given the minimum supply of water, with the exception of the north soil test, series B, when it was slightly less than the amount recovered with the maximum water.

A comparison of series C, having potash residue, with series B and series D, both with added potash, shows a marked luxury consumption of that substance. The yields of both seed and total crop in series C, (Table 3) are practically equal to those in series B and D, but the amounts of potash recovered (Table 4) are notably less. Apparently the residual potash of the soil of series C was sufficient for the needs of the soy-bean plants.

The marked increase in the amount of potash recovered by both millet and soy beans from series C in the second season, when nitrogen and phosphoric acid had been supplied more liberally, indicates that the lack of these substance in the first season limited the effect of the potash.

SUMMARY

Pot experiments were conducted in which Japanese millet and soy beans were grown with varied amounts of potash and of water supplied to the soil, in order to ascertain the relation of the water supply to the availability of the soil potash and the extent to which additions of soluble potash would overcome the lack of water.

Four series of pots were prepared with relation to potash. Series A contained soil that had received no potash fertilizers for 30 years.

Series B had potash added to the soil of A. Series C contained soil that had received annual applications of potash fertilizers for 30 years. Series D had potash added to the soil of C.

Each series received maximum, medium, and minimum supplies of water, based on the water-holding capacity of the soil. The soils with the least water were more like a naturally dry soil than a soil during a drought because the limited quantities of water were added at short intervals.

The experiments were conducted two seasons. In the first season, the supplies of nitrogen, phosphoric acid, and potash were at the rate used in the field from which the soils were obtained. The supplies of these for the second season were much increased as there were indications that the first year's allowance was too small.

Both millet and soy beans on the soil without potash for years gave yields which varied directly as the supplies of water. The percentages of potash in the crops and the quantities by weight indicated that the soil potash had a low solubility and the amount available for crops was dependent on the supply of water.

The millet crops when supplied with available potash were not much affected by lessened supplies of water. The addition of potash increased the production of straw proportionally more than that of seed.

The soy-bean crops were affected by both potash and water supply and in about the same proportion. Potash increased seed production proportionally more than the yield of straw. On the soil without potash for years, soy beans were slow to mature, and analysis of the seeds, pods, and straw indicated that, with the lack of potash, the nitrogen was not translocated freely from the straw and pods to the seeds.

The plentiful supply of available potash in series B and D in the second season gave an increase in absorption of potash by the crops which was proportionally greater than the increase in plant growth accompanying it.

Available potash with the minimum supply of water enabled millet in the second season to produce 92 gm. of seed and 112 gm. of straw, while without added potash but with abundant water it yielded 75 gm. of seed and 64 gm. of straw. Soy beans under parallel conditions produced 74 gm. of seed with the added potash and 45 gm. without it, but the straw was affected by the water supply.

CONCLUSION

The natural potash of the soils used in these pot experiments had a low solubility, and both millet and soy beans obtained their supplies of potash from them in direct proportion to the supplies of water. The addition of potash to the other fertilizers increased the concentration of that substance in the soil solution and the millet was then nearly indifferent to the varied amounts of water supplied to it. Soy beans were about equally affected by both potash and water. For testing the availability of potash in a soil, millet appeared to be a better crop than soy beans because the millet was less affected by variations in the water supply.

The results of the experiment are in accord with the law of the minimum.

THE INFLUENCE OF POSITION OF CATTLE, AS TO STANDING AND LYING, ON THE RATE OF METABOLISM [1]

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INTRODUCTION

In 1924 Fries and Kriss published from this Institute a paper [2] which described a new method for the computation of the observed heat production to a standard day as to standing and lying, this method depending on the idea, which was also new, that the true difference in the heat production of standing and lying is a difference in the maintenance requirement of net energy, and, therefore, that this difference may be determined directly from the heat production of standing and lying during fast, since the heat production during fast is the measure of the maintenance requirement of net energy.

The significance of this matter is that in any determination of the effects of the quantity or kind of feed on the heat production of an animal it is necessary consistently to recognize its status as to activity, so that the characteristic effects of the feed on the heat production will be accurately revealed and will not be obscured by the effects of differences in muscular movement.

In this relation it is possible to compensate for differences in time spent in the standing and the lying positions, which constitute a major factor among the voluntary activities of the animal. As to other voluntary activities, the conditions of experimentation, especially as to method of confinement of the subject, serve prominently to affect and to limit their extent and kinds.

Fortunately these other movements, which are not measured in the procedure employed, are not commonly of such extent as to introduce serious confusion into the determination of the heat production as related to the treatment of the animal.

Since the publication of the above-mentioned paper of Fries and Kriss the writers have hoped that this was a solved problem; and it has been the custom in the determination of the net-energy values of feeds at this institute to compute the heat production, as observed, to standard days as to standing and lying, from the time spent by the animal in these positions, and the factor of Fries and Kriss—representing the difference in heat production in these two positions.

As this method has been used, however, for computing the observed heat production in series of experimental days to standard days, as to standing and lying, the agreement of the heat production for the several days in series has not always been much improved, as it presumably would be (unless obscured by experimental errors) if the basis of the computation were satisfactory.

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[1] Received for publication Aug. 8, 1927; issued December, 1927.

[2] FRIES, J. A., and KRISS, M. METABOLISM OF CATTLE DURING STANDING AND LYING. Amer. Jour. Physiol. 71: 60-83. 1924.

On this account the writers have been obliged to consider this a continuing problem, and after having been on the lookout for some time for a new avenue of approach, they are now able to make a further contribution on the subject, revealing the problem in a new light, and having the effect very extensively to increase the magnitude of the factor representing the difference in energy cost of standing and lying.

The quantitative determination of the comparative energy metabolism during standing and lying, either by direct or indirect calorimetry, presents many difficulties, and the results obtained by different investigators vary widely, in accord with differences in fundamental conceptions involved, and under the influence of different conditions of experimentation, especially as to size of animal and plane of nutrition. Thus Hagemann,[3] in two experiments with steers, reported increases of 28 per cent and 30 per cent in the total heat production of standing as compared with lying. Dahm,[4] working in Zuntz's laboratory, and by Zuntz's method, found an increase of only 8 per cent in the respiratory excretion of carbon dioxide by a young bull, when standing as compared with lying. Klein [5] studied the respiratory exchange of a steer, during standing and lying, by means of a tracheal cannula, and found an increase of 20.7 per cent in the heat production of standing as compared with lying. Armsby and Fries [6] calculated that in 37 published experiments with steers the increase in the directly determined heat production during standing as compared with lying varied from a minimum of 28.3 per cent to a maximum of 64.5 per cent, averaging 41.4 per cent; and they stated that a considerable number of other experiments, unpublished at that time, gave similar results. Armsby and Fries also reported results of six experiments with a steer in which the carbon dioxide and water vapor, as well as the heat produced, were determined separately for intervals of standing and lying, and showed increases in carbon dioxide elimination during standing as compared with lying varying from 20.4 to 35.1 per cent, with corresponding increases in heat production varying between 32.3 and 40.0 per cent.

In all of the experiments referred to above the animals received feed.

Recently Fries and Kriss [7] pointed out certain instrumental errors and imperfections in the method used by Armsby and Fries to separate the directly measured heat production between intervals of standing and lying, especially in feeding periods, and by an indirect computation concluded that a fasting cow, weighing 400 kgm., gave off while standing 26.3 Calories more per hour than while lying, this increase being equal to 9.8 per cent of the total heat production.

This figure was derived from observations on a single cow, but under conditions regarded at the time as unusually favorable. In the light of the present paper, however, which is based upon much

[3] HAGEMANN, O. DAS RESPIRATIONS-CALORIMETER IN BONN UND EINIGE UNTERSUCHUNGEN MIT DEMSELBEN BEI ZWEI RINDERN UND EINEM PFERDE. Landw. Jahrb. 41 (Ergänzbd. 1): 61, 128. 1911.

[4] DAHM, K. DIE BEDEUTUNG DES MECHANISCHEN TEILS DER VERDAUUNGSARBEIT FÜR DEN STOFFWECHSEL DES RINDES. Biochem. Ztschr. 28: 457–503. 1910.

[5] HEIDE, R. VON DER KLEIN, and ZUNT, N. RESPIRATIONS UND STOFFWECHSELVERSUCHE AM RINDE ÜBER DEN NÄHRWERT DER KARTOFFELSCHLEMPE UND IHRER AUSGANGSMATERIALIEN. Landw. Jahrb. 44: [765]-832. 1913.

[6] ARMSBY, H. P., and FRIES, J. A. INFLUENCE OF STANDING OR LYING UPON THE METABOLISM OF CATTLE. Amer. Jour. Physiol. 31: 245–253. 1913.

[7] FRIES, J. A., and KRISS, M. Op. cit.

more extensive data, and improved technic and computations, the problem presented by the instrumental lag, in the study by Fries and Kriss, appears not to have been successfully handled; and it seems imperative, therefore, to reconsider the whole subject.

CONDITIONS OF EXPERIMENTATION

The subjects of this investigation were two Aberdeen Angus steers designated as Nos. 36 and 47, their live weights being 468 and 479 kgm., respectively.

In the spring of 1927 each of these steers was subjected to a fast, steer No. 36 for a period of 7 days, and steer No. 47 for a period of 6 days. During the last four days of these fasting periods the animals were subjected to continuous respiration calorimetric study.

For the purpose of this investigation, in addition to the usual heat measurement, samples of the air coming from the calorimeter chamber were taken at intervals of 15 minutes, during two of the four calorimeter days, for the determination of CO_2 production. In case of steer No. 47 such samples were taken during the second and third of the calorimeter days—these being the fourth and fifth days of fast. With steer No. 36 such samples were taken during the last two calorimeter days—the sixth and seventh days of fast. Carbon dioxide was determined in these air samples by means of a Sondén apparatus.

The rate of ventilation was practically constant during these experiments. Throughout their course record was kept of the time of all changes of position of the animals, as to standing and lying. Instead of considering the results, as usual, for experimental days of 24 hours, however, the data involved in this study are discussed in 12-hour subperiods.

The animals were allowed to change position at will, except during one interval of three and a half hours in the first half of the last day (subperiod 7) in the case of steer No. 36. During this interval the steer was prevented from lying down by means of a chain, purposely connected across the calorimeter stall beneath the animal's body, but sufficiently low so that the animal could not rest upon it. This was done to make possible the collection of air samples certainly and accurately representative of the standing position, since both animals changed position rather frequently and spent much more time lying than standing. This procedure satisfactorily served the intended purpose. The animal was rather quiet, and made no apparent effort to lie down while the chain was connected.

DETERMINATION OF THE CARBON DIOXIDE PRODUCTION FOR STANDING AND LYING

It was realized, from the beginning of this study, that samples of air drawn from the chamber soon after the animal had changed position would not be accurately representative of the new position, on account of instrumental lag. The air capacity of the chamber, with the animal inside, was calculated to be approximately 11,000 liters, and the passage of air through the chamber was at the rate of 450 liters per minute. At this rate of ventilation, therefore, about 25 minutes were required to pump through the chamber a volume of air equal to the chamber capacity. The all-important question as to

how much more than 25 minutes was required for the air in the chamber to reach a composition representative of the position of the animal was left to be answered by the experimental data.

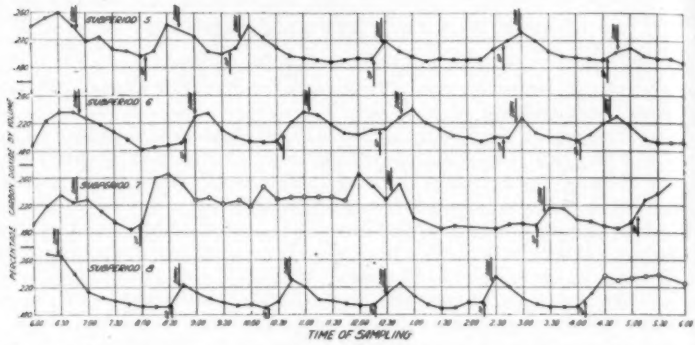

FIG. 1.—Influence of standing and lying on the carbon dioxide content of the air coming from the respiration chamber; steer No. 36. (Stars represent lying position; circles, the standing position)

It was anticipated that the analysis of the air samples taken at frequent and regular intervals (15 minutes) would make possible the tracing of the instrumental lag, and the determination of the time of

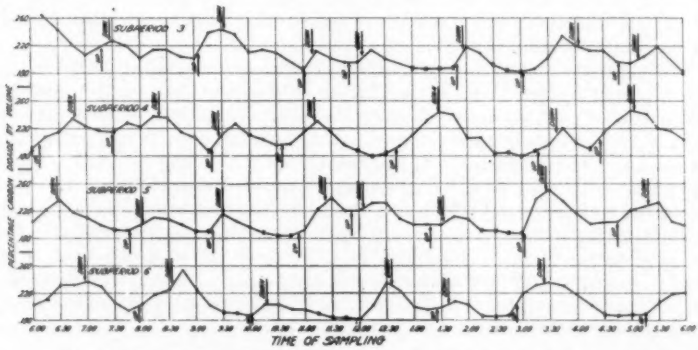

FIG. 2.—Influence of standing and lying on the carbon dioxide content of the air coming from the respiration chamber; steer No. 47. (Stars represent lying position)

operation necessary for the reduction of this lag to a negligible factor. For the accomplishment of this purpose CO_2 was determined in the 15-minute samples, as indicated in Table 1, and the results were plotted. (Figs. 1 and 2.)

TABLE 1.—*Carbon dioxide content of outcoming air as affected by the position of fasting steers; experiment No. 238*

STEER NO. 47

Date	Time of sampling	Position of animal, and time of change of position	Carbon dioxide by volume
	a. m.	*a. m.*	*Per cent*
Subperiod 3	6.00	Up 5.33	0.264
April 20 1927	6.15	do	.265
	6.30	Down 6.18	.243
	6.45	do	.222
	7.00	do	.206
	7.15	do	
		Up 7.18	
	7.30	Down 7.26	.227
	7.45	do	.220
	8.00	do	.202
	8.15	do	.214
	8.30	do	.214
	8.45	do	.204
	9.00	do	.202
	9.15	Up 9.04	.240
	9.30	do	.245
	9.45	Down 9.33	.236
	10.00	do	.210
	10.15	do	.214
	10.30	do	.210
	10.45	do	.168
	11.00	do	.185
		Up 11.01	
	11.15	Down 11.11	.212
	11.30	do	.202
	11.45	do	.196
	Noon		
	12.00	Up 11.51	.196
	p. m.	*p. m.*	
	12.15	Down 12.05	.214
	12.30	do	.199
	12.45	do	.194
	1.00	do	.188
	1.15	do	.186
	1.30	do	.187
	1.45	do	.187
		Up 1.50	
	2.00	Down 1.56	.218
	2.15	do	.209
	2.30	do	.193
	2.45	do	.184
	3.00	do	.182
	3.15	Up 3.02	.186
	3.30	do	.202
	3.45	do	.234
	4.00	do	.220
	4.15	Down 4.04	.213
	4.30	do	.213
	4.45	do	.196
	5.00	Up, 4.48	.194
	5.15	Down, 5.09	.204
	5.30	do	.218
	5.45	do	
	6.00	do	.182
Subperiod 4	6.15	Up, 6.07	.207
	6.30	do	.215
	6.45	do	.234
	7.00	Down, 6.47	.222
	7.15	do	.217
	7.30	Up, 7.29	.215
	7.45	do	.228
	8.00	do	.222
	8.15	do	.238
	8.30	Down, 8.21	.235
	8.45	do	.215
	9.00	do	.208
	9.15	do	.186
		Up, 9.19	
	9.30	Down, 9.26	.213
	9.45	do	.227
	10.00	do	.211
	10.15	do	.205
	10.30	do	.195
	10.45	Up, 10.36	.198

Date	Time of sampling	Position of animal, and time of change of position	Carbon dioxide by volume
	p. m.	*p. m.*	*Per cent*
Subperiod 4	11.00	Up, 10.36	0.215
	11.15	Down, 11.11	.230
	11.30	do	.215
	11.45	do	.196
	Midnight		
Apr. 21, 1927	12.00	do	.189
	a. m.		
	12.15	do	.180
	12.30	*a. m.*	.184
	12.45	Up, 12.41	.194
	1.00	do	.212
	1.15	do	.231
	1.30	Down, 1.28	.245
	1.45	do	.239
	2.00	do	.206
	2.15	do	.208
	2.30	do	.183
	2.45	do	.186
	3.00	do	.179
	3.15	do	.189
	3.30	Up, 3.18	.197
	3.45	Down, 3.38	.221
	4.00	do	.198
	4.15	do	.190
	4.30	Up, 4.27	.212
	4.45	do	.232
	5.00	do	.246
	5.15	Down, 5.04	.241
	5.30	do	.219
	5.45	do	.216
	6.00	do	.203
Subperiod 5	6.15	Up, 6.07	.222
	6.30	Down, 6.27	.237
	6.45	do	.218
	7.00	do	.210
	7.15	do	.198
	7.30	do	.193
	7.45	do	.191
	8.00	Up, 7.47	.200
	8.15	Down, 8.01	.211
	8.30	do	.208
	8.45	do	.200
	9.00	do	.190
	9.15	do	.190
		Up, 9.19	
	9.30	Down, 9.30	.216
	9.45	do	.204
	10.00	do	.196
	10.15	do	.189
	10.30	do	.183
	10.45	do	.183
	11.00	Up, 10.54	.192
	11.15	do	.225
	11.30	Down, 11.23	.240
	11.45	do	.220
	Noon		
	12.00	Up, 11.52	.221
	p. m.	*p. m.*	
	12.15	Down, 12.04	.233
	12.30	do	.233
	12.45	do	.207
	1.00	do	.201
	1.15	do	.200
	1.30	Up, 1.19	.200
	1.45	Down, 1.33	.212
	2.00	do	.207
	2.15	do	.193
	2.30	do	.191
	2.45	do	.189
	3.00	do	.189
	3.15	Up, 3.02	.238
	3.30	Down, 3.26	.252
	3.45	do	.234

TABLE 1.—*Carbon dioxide content of outcoming air as affected by the position of fasting steers; experiment No. 238*—Continued

STEER NO. 47—Continued

Date	Time of sampling	Position of animal, and time of change of position	Carbon dioxide by volume	Date	Time of sampling	Position of animal, and time of change of position	Carbon dioxide by volume
	p. m.	*p. m.*	*Per cent*		*p. m.*	*p. m.*	*Per cent*
Subperiod 5	4.00	Down 3.26	0.216	Subperiod 6	11.30	Down 10.16	0.184
	4.15	do	.201		11.45	do	.184
	4.30	do	.204		*Midnight*		
	4.45	Up, 4.45	.204		12.00	do	.181
	5.00	do	.221	Apr. 22 1927	*a. m.*	*a. m.*	
	5.15	do	.226		12.15	Up, 12.01	.202
	5.30	Down, 5.19	.232		12.30	do	.236
	5.45	do	.204		12.45	Down, 12.35	.224
	6.00	do	.199		1.00	do	.201
Subperiod 6	6.15	Up, 6.07	.212		1.15	do	.196
	6.30	do	.232		1.30	Up, 1.26	.199
	6.45	do	.232		1.45	Down, 1.38	.208
	7.00	Down, 6.56	.238		2.00	do	.205
	7.15	do	.229		2.15	do	.186
	7.30	do	.206		2.30	do	.186
	7.45	do	.194		2.45	do	.187
	8.00	Up, 7.56	.202		3.00	Up, 2.54	.220
	8.15	do	.219		3.15	do	.232
	8.30	do	.225		3.30	Down, 3.24	.237
	8.45	Down, 8.33	.254		3.45	do	.231
	9.00	do	.225		4.00	do	
	9.15	do	.202		4.15	do	.202
	9.30	do	.192		4.30	do	.188
	9.45	do	.191		4.45	do	.187
	10.00	do	.186		5.00	do	.188
	10.15	Up, 10.01	.204		5.15	Up, 5.15	.189
	10.30	Down, 10.16	.204		5.30	do	.207
	10.45	do	.196		5.45	do	.219
	11.00	do	.196		6.00	do	.221
	11.15	do	.190				

STEER NO. 36

	a. m.	*a. m.*			*p. m.*	*p. m.*	
May 5, 1927, subperiod 5	6.00	Up, 5.17	0.240	May 5, 1927, subperiod 5	2.15	Down 12.26	0.192
	6.15	do	.252		2.30	do	.209
	6.30	do	.260		2.45	Up, 2.41	.219
	6.45	do	.238		3.00	Down, 2.58	.233
	7.00	Down, 6.50	.217		3.15	do	.220
	7.15	do	.224		3.30	do	.204
	7.30	do	.206		3.45	do	.198
	7.45	do	.205		4.00	do	.196
	8.00	do	.196		4.15	do	.194
	8.15	Up, 8.06	.204		4.30	do	.193
	8.30	do	.244		4.45	Up, 4.35	.204
	8.45	Down, 8.43			5.00	Down, 4.46	.210
	9.00	do	.226		5.15	do	.198
	9.15	do	.205		5.30	do	.194
	9.30	do	.199		5.45	do	.195
	9.45	Up, 9.39	.208		6.00	do	.188
	10.00	Down, 9.50	.242	Subperiod 6	6.15	Up, 6.03	.224
	10.15	do	.224		6.30	do	.237
	10.30	do	.210		6.45	do	.235
	10.45	do	.197		7.00	Down, 6.52	.227
	11.00	do	.195		7.15	do	.219
	11.15	do	.192		7.30	do	.207
	11.30	do	.187		7.45	do	.196
	11.45	do	.192		8.00	do	.182
	Noon				8.15	do	.186
	12.00	do	.194		8.30	do	.189
	p. m.				8.45	do	.191
	12.15	do	.192			Up, 8.49	
		p. m.			9.00	Down, 9.00	.231
		Up, 12.18			9.15	do	.235
	12.30	Down, 12.26	.219		9.30	do	.210
	12.45	do	.204		9.45	do	.200
	1.00	do	.196		10.00	do	.194
	1.15	do	.190		10.15	do	.193
	1.30	do	.194		10.30	do	.193
	1.45	do	.192		10.45	Up, 10.37	.220
	2.00	do	.192		11.00	do	.236
					11.15	Down, 11.06	.232
					11.30	do	.218
					11.45	do	.206

TABLE 1.—*Carbon dioxide content of outcoming air as affected by the position of fasting steers; experiment No. 238*—Continued

STEER NO. 36—Continued

Date	Time of sampling	Position of animal, and time of change of position	Carbon dioxide by volume	Date	Time of sampling	Position of animal, and time of change of position	Carbon dioxide by volume
	Midnight	*p. m.*	*Per cent*		*p. m.*	*p. m.*	*Per cent*
May 6, 1927	12.00	Down, 11.06	0.204	Subperiod 7	3.15	Down, 12.36	0.192
	12.15	do	.211			Up, 3.16	
					3.30	Down, 3.24	.218
	a. m.	*a. m.*			3.45	do	.216
	12.30	Up, 12.23	.212		4.00	do	.200
	12.45	do	.229		4.15	do	.197
	1.00	Down, 12.47	.240		4.30	do	.191
	1.15	do	.221		4.45	do	.187
	1.30	do	.212		5.00	do	.195
	1.45	do	.203		5.15	Up, 5.07	.228
	2.00	do	.200		5.30	do	.236
	2.15	do	.194		5.48	do	.254
	2.30	do	.201	Subperiod 8	6.15	do	.269
	2.45	Up, 2.40	.198		6.30	Down, 6.27	.266
	3.00	Down, 2.54	.228		6.45	do	.241
	3.15	do	.206		7.00	do	.214
	3.30	do	.199		7.15	do	.206
	3.45	do	.200		7.30	do	.201
	4.00	do	.194		7.45	do	.195
	4.15	Up, 4.04	.204		8.00	do	.193
	4.30	do	.220		8.15	do	.193
	4.45	Down, 4.37	.230		8.30	do	.192
	5.00	do	.214			Up, 8.32	
	5.15	do	.196		8.45	Down, 8.40	.224
	5.30	do	.192		9.00	do	.214
	5.45	do	.193		9.15	do	.204
	6.00	do	.193		9.30	do	.198
Subperiod 7	6.15	Up, 6.02	.219		9.45	do	.194
	6.30	do	.236		10.00	do	.195
	6.45	do	.225		10.15	do	.190
	7.00	Down, 6.48	.220		10.30	Up, 10.19	.199
	7.15	do	.212		10.45	Down, 10.43	.231
	7.30	do	.197		11.00	do	.221
	7.45	do	.185		11.15	do	.203
	8.00	Up, 7.58	.194		11.30	do	.201
	8.15	do	.260		11.45	do	.197
	8.30	do	.266				
	8.45	do	.252		*Midnight*		
	9.00	do	.227	May 7, 1927	12.00	do	.194
	9.15	do	.231				
	9.30	do	.222		*a. m.*		
	9.45	do	.227		12.15	do	.195
	10.00	do	.218			*a. m.*	
	10.15	do	.249			Up, 12.17	
	10.30	do	.228		12.30	Down, 12.28	.212
	10.45	do	.232		12.45	do	.226
	11.00	do	.232		1.00	do	.208
	11.15	do	.232		1.15	do	.195
	11.30	do	.231		1.30	do	.190
	11.45	do	.226		1.45	do	.191
					2.00	do	.199
	Noon				2.15	do	.198
	12.00	do	.266			Up, 2.17	
					2.30	Down, 2.26	.236
	p. m.				2.45	do	.221
	12.15	do	.249		3.00	do	.204
	12.30	do	.228		3.15	do	.197
		p. m.			3.30	do	.193
	12.45	Down, 12.36	.252		3.45	do	.192
	1.00	do	.202		4.00	do	.192
	1.15	do			4.15	Up, 4.08	.213
	1.30	do	.186		4.30	do	.237
	1.45	do	.190		4.45	do	.230
	2.00	do			5.00	do	.234
	2.15	do			5.15	do	.236
	2.30	do	.186		5.30	do	.238
	2.45	do	194		5.45	do	
	3.00	do	.194		6.00	do	.226

The several graphs indicate clearly the course of metabolism during standing and lying as affected by the instrumental lag. They show that after a change of position about an hour was required for the CO_2 of the outcoming air to reach essentially a constant. Such

parts of the graphs as appear to be practically beyond the influence of the instrumental lag have been designated by special marks, those representing the lying position being starred, while those representing the standing position are marked by circles. The corresponding percentages of CO_2 for standing and lying have been averaged, and are presented in Tables 2 and 3.

Since in six of the eight 12-hour subperiods the animal did not stand for sufficiently long intervals to permit the direct determination of the CO_2 production for standing, this was obtained indirectly through a computation by difference. This computation, in general terms, consisted in subtracting the CO_2 production as determined for the entire time spent in the lying position from the total CO_2 produced during the 12-hour subperiod, and relating the remainder to the time spent by the animal in the standing position during the same subperiod. This computation, the results of which are presented in Table 4, was performed as indicated below:

(*a*) The total CO_2 in the outcoming air was obtained by multiplying the average percentage of CO_2 of all samples analyzed, by means of the Sondén apparatus, by the quantity of the total ventilation.

(*b*) To the total CO_2 obtained in (*a*) a residual correction, derived from a special 15-minute continuous aspirator sample of the outcoming air, was applied, to correct for the difference in composition of the air in the respiration chamber at the end as compared with the beginning of a 12-hour subperiod.

(*c*) From the total corrected CO_2 in the outcoming air was subtracted the total CO_2 of the ingoing air, as determined gravimetrically in a continuous sample. The remainder is the total CO_2 produced by the animal.

(*d*) The total outcoming air during lying was computed from the total ventilation, in proportion to the time spent in the lying position.

(*e*) The liters of CO_2 in the outcoming air during lying were obtained by multiplying the average per cent of CO_2 for lying, as in Table 2, by the volume of outcoming air during lying, as obtained in (*d*).

(*f*) The liters of CO_2 in the outcoming air during standing were computed by subtracting the liters of CO_2 in the outcoming air during lying, as obtained in (*e*), from the total CO_2 in the outcoming air, as obtained in (*b*).

(*g*) The quantities of CO_2 in the ingoing air during lying and standing, respectively, were computed from the total CO_2 of the ingoing air in proportion to the time spent in the lying and the standing positions.

(*h*) The CO_2 produced during lying and standing, respectively, was obtained by subtracting from the CO_2 in the outcoming air for lying and standing (*e* and *f*) the corresponding quantities of CO_2 in the ingoing air.

(*i*) The CO_2 produced per hour, during lying and standing, respectively, was calculated from the corresponding totals, as obtained in (*h*), and the durations of time of lying and standing represented.

The data obtained in (*f*), (*h*), and (*i*), for standing, however, include the effect of the effort of rising and of lying down. The CO_2 produced during standing, excluding this effect of the effort of rising and lying down, was obtained by the following calculations:

(*j*) The ratio of heat to CO_2 was computed by dividing the number of Calories of observed heat production by the liters of CO_2 produced, as obtained in (*c*).

(*k*) The energy required for rising and lying down was computed by the use of Klein's[8] factor of 9.7 Calories per act of rising and lying down, together, by a steer weighing 550 kgm.; this factor, modified directly according to the weight of the animal, being multiplied by the number of times the animal arose and lay down again.

(*l*) The CO_2 required for rising and lying down was obtained by dividing the Calories required for rising and lying down by the heat-CO_2 ratio.

(*m*) The CO_2 produced during standing, excluding the effect of the act of rising and lying down, was computed by subtracting the CO_2 required for rising and lying down from the CO_2 produced during standing, as obtained in (*h*). The same was also computed, per hour, as in (*i*).

(*n*) The CO_2 produced during standing by steer No. 36 in subperiods 7 and 8 was also computed directly by first multiplying the average percentage of CO_2 in the outcoming air during standing, as presented in Table 3, by the volume of the outcoming air during standing, and then subtracting from the product the corresponding quantity of CO_2 of the ingoing air.

DISCUSSION OF RESULTS

In general the results obtained with steer No. 36 may be regarded as more satisfactory than those with steer No. 47, principally because the former changed position less frequently than the latter. The number of intervals of time spent by steer No. 36 in the lying position during a 12-hour subperiod varied from 3 to 6, while in the case of steer No. 47 the number varied from 7 to 8. The intervals spent in one position, therefore, were longer, in most cases, with steer No. 36 than with steer No. 47. This fact afforded a relatively better basis for the determination of the CO_2 production during lying with steer No. 36, as can be seen both in Figures 1 and 2 and in Table 2. It also provided, with this animal, a basis for the direct determination of the CO_2 production during standing, in subperiods 7 and 8 (Table 3), whereas such a determination was not possible with steer No. 47 in any of the subperiods.

The data presented in Table 2 represent the percentages of CO_2 in the outcoming air during lying, with the factor of instrumental lag presumably excluded. If there were also a physiological lag in the adjustment of metabolism to changes of position, this would be indistinguishable, by the method of experimentation employed, from the instrumental lag; and, for the present purpose, a procedure adequate to the elimination of the experimental lag, as observed, may be considered to cover the instrumental lag and a conceivable physiological lag, as well. In this relation, however, the data of Table 2 have a special significance, since they represent the metabolism of the animal adjusted to the lying position.

The average percentages CO_2 in the outcoming air during lying are, with steer No. 36—0.193, 0.192, 0.191, and 0.194, in subperiods 5, 6, 7, and 8, respectively. With steer No. 47 the corresponding values are 0.186, 0.185, 0.189, and 0.187, for subperiods 3, 4, 5, and 6, respectively. There is, therefore, good agreement between the subperiods with both animals. This agreement is significant, because the rate of ventilation was practically constant throughout the experiment.

[8] HEIDE, R. VON DER KLEIN, and ZUNTZ, N. Op. cit.

TABLE 2.—*Percentages of carbon dioxide in outcoming air representing metabolism during lying; experiment No. 238*

Period 11, steer No. 47, fasting			Period 12, steer No. 36, fasting		
Subperiod	Time of sampling	Percentage CO_2	Subperiod	Time of sampling	Percentage CO_2
	a. m.			*a. m.*	
	11.00	0.185	No. 5	8.00	0.196
	p. m.			10.45	.197
No. 3	1.00	.188		11.00	.195
	1.15	.186		11.15	.192
	1.30	.187		11.30	.187
	1.45	.187		11.45	.192
	2.30	.193		*Noon*	
	2.45	.184		12.00	.194
	3.00	.182		*p. m.*	
	6.00	.182		12.15	.192
				1.00	.196
				1.15	.190
				1.30	.194
				1.45	.192
				2.00	.192
				2.15	.192
				4.00	.196
				4.15	.194
				4.30	.193
				5.30	.194
				5.45	.195
				6.00	.188
Average		.186	Average		.193
	p. m.			*p. m.*	
No. 4	9.15	.186	No. 6	6.00	.188
	Midnight			7.45	.196
	12.00	.189		8.00	.182
				8.15	.186
	a. m.			8.30	.189
	12.15	.180		8.45	.191
	12.30	.184		10.00	.194
	2.30	.183		10.15	.193
	2.45	.186		10.30	.193
	3.00	.179		*a. m.*	
	3.15	.189		2.15	.194
	4.15	.190		2.30	.201
				4.00	.194
				5.15	.196
				5.30	.192
				5.45	.193
				6.00	.193
Average		.185	Average		.192
	a. m.			*a. m.*	
No. 5	7.30	.193	No. 7	6.00	.193
	7.45	.191		7.30	.197
	9.00	.190		7.45	.185
	9.15	.190		*p. m.*	
	10.15	.189		1.30	.186
	10.30	.183		1.45	.190
	10.45	.183		2.30	.186
	p. m.			2.45	.194
	2.15	.193		3.00	.194
	2.30	.191		3.15	.192
	2.45	.189		4.15	.197
	3.00	.189		4.30	.191
				4.45	.187
				5.00	.195
Average		.189	Average		.191
				p. m.	
	p. m.		No. 8	7.45	.195
No. 6	9.30	.192		8.00	.193
	9.45	.191		8.15	.193
	10.00	.186		8.30	.192
	11.15	.190		9.45	.194
	11.30	.184		10.00	.195
	11.45	.184		10.15	.190
				11.45	.197

TABLE 2.—*Percentages of carbon dioxide in outcoming air representing metabolism during lying; experiment No. 238*—Continued

Period 11, steer No. 47, fasting			Period 12, steer No. 36, fasting		
Subperiod	Time of sampling	Percentage CO_2	Subperiod	Time of sampling	Percentage CO_2
	Midnight 12.00	0.181		*Midnight* 12.00	0.194
	a. m.			a. m.	
No. 6	2.15	.186	No. 8	12.15	.195
	2.30	.186		1.15	.195
	2.45	.187		1.30	.190
	4.30	.188		1.45	.191
	4.45	.187		2.00	.199
	5.00	.188		2.15	.198
	5.15	.189		3.15	.197
				3.30	.193
				3.45	.192
				4.00	.192
Average		.187	Average		.194

TABLE 3.—*Percentages of carbon dioxide in outcoming air representing metabolism during standing; experiment No. 238*

PERIOD 12, STEER NO. 36, FASTING

Subperiod	Time of sampling	Percentage CO_2	Subperiod	Time of sampling	Percentage CO_2
	a. m.			a. m.	
No. 7	9.00	0.227	No. 8	4.30	0.237
	9.15	.231		4.45	.230
	9.30	.222		5.00	.234
	9.45	.227		5.15	.236
	10.00	.218		5.30	.238
	10.15	.249		6.00	.226
	10.30	.228			
	10.45	.232			
	11.00	.232			
	11.15	.232			
	11.30	.231			
	11.45	.226			
Average		.230	Average		.234

Table 3 gives the percentages of CO_2 in the outcoming air representing the metabolism of the animal while standing, and also exclusive of the influence of the experimental lag. In subperiod 7 the data represent enforced standing, while those of subperiod 8 represent voluntary standing. These data do not exhibit any significant difference between the enforced and the voluntary standing, the average percentage of CO_2 in subperiod 7 being 0.230, and in subperiod 8, -0.234.

The values for CO_2 production per hour, during lying, which appear in Table 4, and which were derived from the data of Table 2, exhibit a very good agreement between the subperiods as well as between the two animals. These values for steer No. 47 in the four subperiods here considered are 42.0, 41.5, 42.5, and 42.1 liters, respectively, averaging 42.0 liters, while for steer No. 36 the corresponding values are 43.3, 43.0, 43.1, and 43.8 liters, averaging 43.3 liters. Steer No. 36 appears to have produced slightly more CO_2 per hour while lying than did steer No. 47. It should be borne in mind that the above values represent the metabolism of the animal during lying, exclusive of the influence of the experimental lag.

TABLE 4.—*Carbon dioxide production during standing and lying; experiment No. 238*

PERIOD 11, STEER NO. 47, FASTING

Item	Subperiod			
	3	4	5	6
Total ventilation.....liters..	324,113.5	324,399.2	322,125.7	322,343.1
CO_2 in outcoming air.....per cent..	0.209	0.211	0.209	0.207
CO_2 in outcoming air.....liters..	677.4	684.5	673.2	667.3
Residual correction.....do....	−6.5	+1.6	−0.4	+3.2
Total CO_2 in outcoming air, corrected.....do....	670.9	686.1	672.8	670.5
Total CO_2 in ingoing air.....do....	98.7	102.7	99.0	97.1
Total CO_2 produced.....do....	572.2	583.4	573.8	573.4
Time spent lying.....minutes..	552	482	562	512
Time spent standing.....do....	168	238	158	208
Outcoming air during lying.....liters..	248,486.7	217,167.2	251,437.0	229,221.8
CO_2 in outcoming air during lying.....per cent..	0.186	0.185	0.189	0.187
CO_2 in outcoming air during lying.....liters..	462.2	401.8	475.2	428.6
CO_2 in outcoming air during standing.....do....	208.7	284.3	197.6	241.9
CO_2 in ingoing air during lying.....do....	75.7	68.8	77.3	69.0
CO_2 in ingoing air during standing.....do....	23.0	33.9	21.7	28.1
CO_2 produced during lying.....do....	386.5	333.0	397.9	359.6
CO_2 produced during standing.....do....	185.7	250.4	175.9	213.8
CO_2 produced during lying, per hour.....do....	42.0	41.5	42.5	42.1
CO_2 produced during standing, per hour.....do....	66.3	63.1	66.8	61.7
Total heat production.....Calories..	3,703.4	3,843.4	3,628.3	3,732.9
Heat: CO_2.....	6.47	6.59	6.32	6.51
Energy required for rising and lying down.....Calories[a]..	63.8	59.5	68.0	59.5
CO_2 required for rising and lying down.....liters..	9.9	9.0	10.8	9.1
CO_2 produced during standing, excluding act of rising and lying down.....liters..	175.8	241.4	165.1	204.7
CO_2 produced during standing, excluding act of rising and lying down, per hour.....liters..	62.8	60.9	62.7	59.0

PERIOD 12, STEER NO. 36, FASTING

Item	Subperiod			
	5	6	7	8
Total ventilation.....liters..	324,129.4	324,392.4	323,776.3	324,486.2
CO_2 in outcoming air.....per cent..	0.208	0.209	0.220	0.212
CO_2 in outcoming air.....liters..	674.2	678.0	[b] 710.4	687.9
Residual correction.....do....	−5.1	+0.2	+6.6	−3.4
Total CO_2 in outcoming air, corrected.....do....	669.1	678.2	717.0	684.5
Total CO_2 in ingoing air.....do....	106.0	106.7	101.2	103.6
Total CO_2 produced.....do....	563.1	571.5	615.8	580.9
Time spent lying.....minutes..	586	560	335	529
Time spent standing.....do....	134	160	385	191
Outcoming air during lying.....liters..	263,805.3	252,305.2	150,645.9	238,407.2
CO_2 in outcoming air during lying.....per cent..	0.193	0.192	0.191	0.194
CO_2 in outcoming air during lying.....liters..	509.1	484.4	287.7	462.5
CO_2 in outcoming air during standing.....do....	160.0	193.8	429.3	222.0
CO_2 in ingoing air during lying.....do....	86.3	83.0	47.1	76.1
CO_2 in ingoing air during standing.....do....	19.7	23.7	54.1	27.5
CO_2 produced during lying.....do....	422.8	401.4	240.6	386.4
CO_2 produced during standing.....do....	140.3	170.1	375.2	194.5
CO_2 produced during lying, per hour.....do....	43.3	43.0	43.1	43.8
CO_2 produced during standing, per hour.....do....	62.8	63.8	58.5	61.1
Total heat production.....Calories..	3,730.4	3,779.9	4,081.8	3,672.0
Heat: CO_2.....	6.62	6.61	6.63	6.32
Energy required for rising and lying down.....Calories..	49.8	49.8	29.1	41.5
CO_2 required for rising and lying down.....liters..	7.5	7.5	4.4	6.6
CO_2 produced during standing, excluding act of rising and lying down.....liters..	132.8	162.6	370.8	187.9
CO_2 produced per hour during standing, excluding act of rising and lying down.....liters..	59.5	61.0	57.8	59.0
CO_2 produced per hour during standing, excluding act of rising and lying down, directly determined.....liters..			53.6	54.6

[a] 8.3 Calories per act of rising and lying down. [b] Corrected for man in chamber by 1.9 L. CO_2.

Two sets of figures for each animal are the results of the computation, by difference, of the CO_2 production during standing. The first set of figures result directly from subtracting the CO_2 production during lying from the total CO_2 produced, and comprise therefore in addition to the CO_2 of standing, the CO_2 produced as a result of the effort of rising and of lying down. Expressed as volumes per hour these are 66.3, 63.1, 66.8, and 61.7 liters, respectively, in the four

subperiods with steer No. 47; and 62.8, 63.8, 58.5, and 61.1 liters in the four subperiods with steer No. 36.

When corrected for the estimated CO_2 required for rising and lying down the above figures become 62.8, 60.9, 62.7, and 59.0 liters, averaging 61.4 liters, for steer No. 47; and 59.5, 61.0, 57.8, and 59.0 liters, averaging 59.3 liters, for steer No. 36. These corrected values represent the metabolism of the animal during standing, excluding the act of rising and lying down, but including the possible physiological effect of adjustment of the metabolism to the changes in position and the various activities of the animal while standing during the experiment. Aside from the uncertainty of the validity of the factor upon which the corrections for rising and lying down were based, the values for CO_2 production during standing, computed by the difference method, have, therefore, a different significance from such values as determined directly.

The direct determination of the CO_2 production during standing was possible only in two subperiods with steer No. 36, namely, subperiods 7 and 8. The values obtained are based on two intervals of standing, the one covering 4 hours and 38 minutes (from 7.58 a. m. to 12.36 p. m.), in subperiod 7, during which the animal was forced to stand; and the other covering only 1 hour and 52 minutes (from 4.08 a. m. to 6.00 a. m.), in subperiod 8, representing voluntary standing. In subperiod 7 the animal produced 53.6 liters of CO_2 per hour while standing, and in subperiod 8, -54.6 liters per hour. The difference between these values is small, especially in consideration of the differences in the unrecorded activities of the animal while standing, and therefore indicates that there was no material difference in the metabolism of the forced and the voluntary standing.

The average of the two directly determined values for CO_2 production during standing is 54.1 liters per hour, and can be compared with the average CO_2 production by the same animal while lying, which is 43.3 liters; both being determined in such manner as to exclude the influence of instrumental and possible physiological lag. The difference is 10.8 liters per hour. In other words, the steer, weighing 468 kgm., produced 10.8 liters of CO_2 per hour more during standing than during lying. Expressed in relation to 100 kgm. live weight, the increase in CO_2 production due to the effort of standing is 2.31 liters per hour.

The computation of this factor from the basis of the live weight of the animal on which it was determined, to another live-weight basis, may be made in accord with the two-thirds power of the live weight, in consideration of the fact that the basal metabolism varies with the surface area (and disregarding the difference between the basal metabolism, which is measured by the fasting and resting heat production, and the maintenance requirement of net energy, which is measured as the 24-hour fasting metabolism with normal activity), or it may be computed directly in accord with the live weight, as above. The difference is not extensive.

The increase in heat production of standing as compared with lying may be computed by multiplying the corresponding increase in CO_2 by the average heat-CO_2 ratio obtained with the same animal,

on the assumption that this ratio, during fast, is the same for standing and lying.

The heat-CO_2 ratios $\left(\frac{\text{Calories of heat}}{\text{liters of } CO_2}\right)$ obtained with steer No. 36 in the four subperiods are 6.62, 6.61, 6.63, and 6.32. The writers are unable to explain the divergence of the value for subperiod 8 from the three other values. The average of the first three is 6.62. If the fourth value is included the average is 6.55.

For the purpose of computing the difference in heat production between standing and lying it makes very little difference (less than 1 Calorie per head per hour) which of the above average heat-CO_2 ratios is used. On the basis of the average ratio (6.55) for all four subperiods with steer No. 36 the increase in heat production for standing as compared with lying was 70.7 Calories (10.8×6.55) per head, or 15.1 Calories per 100 kgm. live weight.

This value is about two and one-fourth times as great as that obtained with cow No. 874 (26.3 Calories per head, the cow weighing 400 kgm.).[9] The discrepancy between the present and the earlier determination is apparently due chiefly to the fact that in the previous study in the computation of the difference in heat production of standing and lying the total heat production was apportioned between the aggregates of intervals of standing and of lying in proportion to the CO_2 observed for these intervals. This assumes, in effect, that there was no instrumental lag affecting the CO_2 of the intervals of standing and lying involved in the computation, and that the CO_2 as observed correctly represented the heat production of these intervals. In the light of the present understanding it is now clear that in the earlier study an extensive instrumental lag, a "carry-over" from the previous interval, whatever the position of the animal, affected the validity of the observed CO_2, as related to the intervals of standing and lying—the effect being, therefore, very materially to reduce the apparent difference in the CO_2 and the heat of standing and lying.

This study will be continued as a feature of the general program of calorimetric investigation of this institute.

SUMMARY

The determination of the energy expenditure of cattle in the standing compared with the lying position, as a basis for the computation of the heat production to a standard day as to standing and lying, has been reconsidered in the light of new evidence of the extent of the experimental lag, affecting the measurements of CO_2 and heat in the use of the respiration calorimeter.

A steer weighing 468 kgm. produced 10.8 liters more CO_2 per hour (2.31 liters per 100 kgm. live weight) during standing than during lying. On the basis of a determined heat-CO_2 ratio of 6.55, the increase of heat production for standing as compared with lying was 70.7 Calories per head, or 15.1 Calories per 100 kgm. live weight, per hour.

[9] FRIES, J. A., and KRISS, M. Op. cit.